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## EXPERIMENTS IN THE CONTROL OF RHIZOCTONIA DAMPING-OFF OF CITRUS SEEDLINGS<sup>1, 2</sup>

R. WEINDLING<sup>3</sup> AND H. S. FAWCETT<sup>4</sup>

### INTRODUCTION

*Rhizoctonia solani* Kühn has been reported from various localities as the most important fungus associated with the damping-off disease of citrus seedlings.<sup>15, 16, 20, 21, 25</sup> During the last four years this fungus has been constantly isolated from diseased seedlings of sweet orange, grapefruit, and sour orange which were collected in seed beds from various sections of southern California. In the present experiments, the high virulence of the pathogen in sterilized and natural soils has been repeatedly verified. There is an indication from field and greenhouse observations that sour orange is not quite so susceptible to the attack of the fungus as sweet orange.

The disease is readily recognized in the seed bed. The dead seedlings with withered leaves usually remain standing about the margins of the bare areas which mark the centers of infection. At the margins of these areas may be found plants with lesions and apparently healthy seedlings with strands of the fungus hanging like spider webs around the base of the stem. In general, nurserymen are able to avoid damping-off or to keep it within reasonable limits by good cultural practices, such as are discussed by Fawcett and Lee.<sup>16</sup> In some seasons and in some sections, however, the losses due to damping-off have been excessive. In 1934, for instance, 50 to 60 per cent of the seedlings in some beds were affected.

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<sup>2</sup> Paper No. 324, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

<sup>3</sup> Technical Assistant in Plant Pathology, Citrus Experiment Station; resigned June 30, 1935.

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In this paper account will be given of two field experiments in which considerable control of damping-off was obtained by acidifying the surface layer of the soil by the application of acid peat moss or aluminum sulfate. Some details of laboratory and greenhouse experiments will also be presented.<sup>(10)</sup> These experiments had been planned primarily to furnish a basis for control methods in the field, but they also threw light on the reasons why control of *Rhizoctonia* damping-off is obtained by acidification of the top soil.

## LABORATORY AND GREENHOUSE EXPERIMENTS IN BIOLOGICAL CONTROL OF DAMPING-OFF

### FACTORS INVOLVED

Hartley and his collaborators<sup>(10, 11)</sup> demonstrated several years ago that saprophytic soil fungi may play a rôle in decreasing damping-off of conifer seedlings. It was suggested that this might be due to their competition in the soil with the pathogenic organisms and perhaps to their antagonistic effects upon these organisms. Since the discovery by Weindling<sup>(20)</sup> of the parasitic action of *Trichoderma* on *Rhizoctonia solani* and other soil fungi, two sets of investigators<sup>(2, 3)</sup> have obtained positive results with *Trichoderma* spp. for biological control of pathogenic soil fungi. In our first experiments of this kind, application of *Trichoderma* had given good protection to seedlings against *Rhizoctonia* in some cases and little or none in others. These contradictory results were clarified later by a research in the fundamental aspects of the interaction between *Trichoderma* and *Rhizoctonia*. It was shown<sup>(20)</sup> that the parasitic action of the strain of *Trichoderma* used is favored by a strongly acid reaction of the culture medium, and decreases as the medium becomes less acid. Similar effects were obtained in our laboratory experiments with soils autoclaved at 120° C three times for a period of 1 hour each.

Sterilized soils have been used by numerous investigators to demonstrate that plant diseases caused by certain soil-borne plant pathogens may be prevented or reduced by inoculating these soils with antagonistic organisms or with portions of unsterilized soil.<sup>(12, 18, 20)</sup> It has been claimed that the development of fungi in sterilized soil is entirely unlike their growth in natural soil, and that it is much more comparable to artificial growth in culture media.<sup>(17)</sup> Nevertheless, the data from our work with sterilized soil proved to be of value as a basis for planning and interpreting the work with nonsterilized soil.

## EFFECT OF pH AND OTHER FACTORS IN LABORATORY EXPERIMENTS

The following are representative of this type of experiment. As a rule, each treatment was given to three containers. Since inoculation with *Trichoderma* alone did not differ from the controls in which neither *Rhizoctonia* nor *Trichoderma* was used, this treatment was omitted in most of the later experiments. Usually the number of seeds planted was twelve per container.

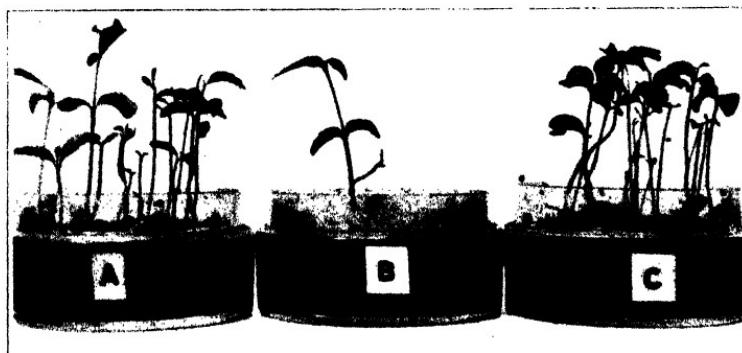


Fig. 1.—Sweet-orange seedlings in sterilized soil. *A*, Check; *B*, *Rhizoctonia* inoculated in soil layer in bottom of jar; *C*, *Rhizoctonia* as in *B*, plus *Trichoderma* in top layer of peat.

In the first test, petri dishes containing peat moss (pH 4.0) were autoclaved and sown with citrus seeds taken aseptically from the fruits. When *Rhizoctonia* alone was inoculated, all germinating seedlings were killed. But if *Trichoderma* spores were at the same time mixed with the peat or sprayed onto the seed, none of the seedlings were attacked.

In another experiment the acid peat moss was adjusted to a series of pH values by the addition of potassium hydroxide. Portions of peat of each acidity were sterilized in pint Mason jars. At the original acidity (pH 4.0) the protection of the seedlings from *Rhizoctonia* by adding *Trichoderma* was as good as in the first test, previously described. At pH 4.5 to 4.7, only 68 per cent control was obtained, and at pH 5.4 to 5.6 and at higher pH values, the loss due to rotting of the germinating seed and to damping-off was as complete as with *Rhizoctonia* alone. In another set with a weaker inoculation of *Rhizoctonia*, the *Trichoderma* protected 70 per cent of the seedlings at pH 4.5 and 50 per cent at pH 5.7 to 6.1, but none at neutral reaction.

Figure 1 shows an experiment with seedlings in glass jars. A mixture of sand and soil (pH 6.5) was placed in the bottom of the jars and this

was covered with an inch of peat. After sterilization, *Rhizoctonia* was inoculated in the bottom soil. The seeds and *Trichoderma* were placed in the peat. In the jars with *Rhizoctonia* alone 90 per cent of the seedlings were killed before emergence; the rest damped-off later. The dishes inoculated with *Rhizoctonia* and with spores of a pigmented culture of *Trichoderma* showed 40 and 50 per cent healthy seedlings, while a culture of *Trichoderma* with a coconut-like odor gave 78 and 95 per cent control.<sup>5</sup>

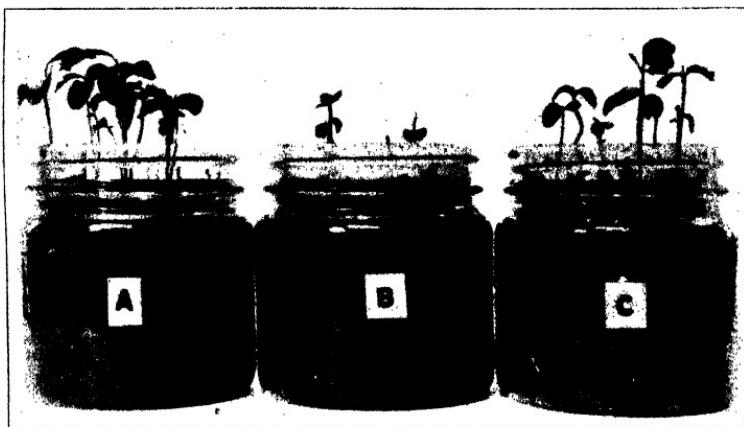


Fig. 2.—Sweet-orange seedlings in nonsterilized soil. *A*, Check; *B*, *Rhizoctonia* inoculated in soil layer in bottom of jar; *C*, *Rhizoctonia* as in *B*, plus *Trichoderma* in top layer of peat.

An experiment similar to the last one mentioned is represented in figure 2. However, these jars with soil and peat were not sterilized. The seed was covered with a mixture of sand and peat. The spores of *Trichoderma* were suspended in a 1 per cent glycerine solution, on which *Trichoderma* grows much better than does *Rhizoctonia*. This fact was established in an experiment in which all other carbon sources tested produced a much denser growth of *Rhizoctonia* than of *Trichoderma*. In *Trichoderma*-inoculated jars, 92 per cent of the seedlings were not affected by damping-off. In the jars with *Rhizoctonia* alone, 22 per cent of the seedlings survived. Abundant sporulation of *Trichoderma* was found, however, on the soil in these jars. A similar effect was noted in an experiment with sterilized acid peat, where insufficient care had been taken to prevent the jars with *Rhizoctonia* alone from being contaminated by the air-borne spores of *Trichoderma*. If other organisms were

<sup>5</sup> The first percentage in each case refers to soil inoculation with *Trichoderma*, the second to seed inoculation.

kept away from sterilized soils, *Rhizoctonia* inoculation would always result in the death of all seedlings, mostly before emergence.

In the work reported thus far, the jars were covered and placed in an incubator at 27° C until the seeds had germinated. Then they were uncovered and held in the laboratory at room temperature. The *Rhizoctonia* was grown on wheat bran for 2 to 4 weeks, 1 to 3 cc of bran being added to the soil; the amount varied with the size of the containers used in these and the following experiments. The spore suspension of *Trichoderma* was prepared from cultures of the fungus that had grown for 10 to 20 days on glucose-potato agar. In some of the work, cultures of *Trichoderma lignorum* producing a coconut-like odor and cultures of *T. koningi* were used in addition to the pigmented cultures of *T. lignorum*. The hydrogen-ion concentrations were determined by the method proposed by Hissink.<sup>(23)</sup>

#### EFFECT OF pH AND OTHER FACTORS IN GREENHOUSE EXPERIMENTS

The acid peat moss had proved to be a very favorable medium for *Trichoderma*. It was used, therefore, in experiments in the greenhouse, carried out to develop methods suitable for preventing the damping-off disease under seed-bed conditions. In general, the plan was to place a sandy loam of about neutral reaction in the bottom of pots or flats, and to inoculate this soil with the *Rhizoctonia* at one spot or several. The soil was covered with a layer of peat moss which served as a bed for the seed. Grapefruit seeds were used most frequently, but sweet and sour orange were used in some cases. The number of seeds used was 25 per 5-inch pot, and 250 to 300 per flat of 2 square feet. The *Trichoderma* spore suspension was added to the peat. The controls consisted of containers with surface layers of soil or peat, *Rhizoctonia* alone being inoculated, or no fungus at all. A digest is here presented of results of a large number of experiments made in an attempt to find the best methods of procedure.

Nearly complete protection from the disease was obtained where a layer of peat, ¾-inch deep and having a reaction of pH 4.0, was placed on the soil, and when the seed was covered with an additional ¾-inch layer of peat moss inoculated with *Trichoderma*. However, when the seed was placed on the soil and covered with sand, all or nearly all of the germinating seedlings were killed by the *Rhizoctonia*, even if *Trichoderma* spores were added in abundance. The reaction of the soil was pH 6.4 to 6.7, that of the sand, pH 7.3 to 7.8. Where peat was used in the containers inoculated with *Rhizoctonia* alone, considerable damping-off sometimes developed; in other cases, however, the disease affected very few seedlings. In the above cases when only a few seedlings were affected by the introduction of *Rhizoctonia* alone with peat, *Trichoderma* could

always be easily isolated from the containers, even if they were kept as far as possible from soil purposely inoculated with this fungus. Thus, *Trichoderma* spp., even if not purposely introduced, seem to represent a group of organisms which at acid reactions of the soil prevent the *Rhizoctonia* from becoming fatal to the seedlings.

The use of any mixture of the peat with sand or soil gave poorer control than with the straight peat. It was with the mixtures of peat and

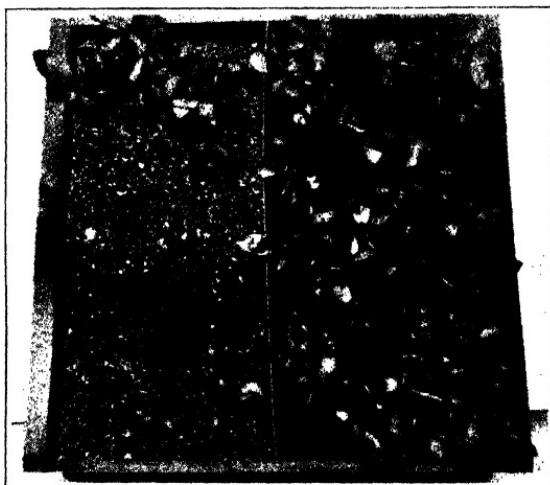


Fig. 3.—At left, grapefruit seedlings in natural soil. At right, soil cover replaced by peat 1 inch deep. Both sides inoculated with *Rhizoctonia* in subsoil at lower end of flat.

sand, however, that biological control with *Trichoderma* could be demonstrated in some experiments. In one of them the seed was covered with a mixture of peat and sand (initial pH 4.5 to 4.8). From 25 to 40 per cent control was obtained from inoculation with *Trichoderma*, inoculation with *Rhizoctonia* alone producing complete loss.

In general, the control of the disease seemed to increase with increasing acidity of the peat mixture. Changes in reaction of the soils were followed over the period of damping-off, that is, for about 14 weeks, by testing samples with the quinhydrone electrode. With smaller percentages of peat in the mixtures, the change towards a neutral reaction was, of course, more rapid. But even in the flats and pots with peat alone there was a gradual decrease of acidity, probably due to the alkalinity of the irrigation water.

Results similar to those reported thus far were also obtained with seedlings inoculated when 6 to 8 weeks old. In figure 3 is shown an example

of this type of experiment. On one side of the flat the upper inch layer of soil was replaced by *Trichoderma*-infested peat, simultaneously with the introduction of the *Rhizoctonia* inoculum in the subsoil at both sides of the partition at the lower end of the flat.



Fig. 4.—Sour-orange seedlings in natural soil. In pots *B* and *C*, the surface inch of the soil was replaced by the same soil treated with aluminum sulfate, simultaneously with *Rhizoctonia* inoculation. Initial reaction of the soil-surface layers: Pot *A*, pH 7.3; Pot *B*, pH 4.9; Pot *C*, pH 4.0.

In other experiments, acidification of the top layer of the soil was attempted by applying aluminum sulfate, which has been frequently employed as a quick and convenient means of soil acidification.<sup>6,10,20</sup> Figure 4 shows the effect on seedlings of *Rhizoctonia* inoculation made at the same time that the upper inch of a slightly alkaline soil was replaced with soil treated with aluminum sulfate to give pH 4.9 to pot *B* and pH 4.0 to pot *C*.

The same soils were also used as surface layers in flats and pots in which seeds were sown. At pH 7.3 there was a total loss, while complete control of the disease was secured at pH 4.0, and only partial control at pH 4.9. Similar results were obtained in each pH series, three pots

\* The writers are indebted to D. E. Bliss, Citrus Experiment Station, for advice regarding methods of applying aluminum sulfate.

being inoculated with *Rhizoctonia* alone, and three with *Rhizoctonia* and *Trichoderma*. Mycelium of *Rhizoctonia* was reisolated easily from the top soil of the pots that had not received *Trichoderma* and which had a reaction of pH 7.3. It was also found in some cultures from soils of pH 4.9, but not at pH 4.0. In these two soils, especially in the more acid one, *Trichoderma* and other common soil molds, such as *Mucor*, *Penicillium*, and *Fusarium*, were found to be very active. The method of Waksman<sup>(27)</sup> to determine fungi growing actively in the soil was employed in these tests. Corn meal and an agar medium containing 1 per cent glycerine only were found rather useful for these isolations.

An attempt was also made to determine the influence of variations in soil temperature on the control obtained by acidifying the surface layer of the soil. In soil-temperature tanks<sup>(28)</sup> at five temperatures between 18° C and 35° C, two pots were covered with the original soil and two pots with the original soil treated with aluminum sulfate to give a pH of 4.0 to the top 1½ inches. Three-week-old grapefruit seedlings were planted and were severely injured by the acidified soil. They were replaced by six-week-old sour-orange seedlings, a few of which were also planted in the nonacidified pots in addition to the grapefruit seedlings. All pots were uniformly inoculated with *Trichoderma* at the time of planting and with *Rhizoctonia* 3 weeks later. Complete control of the disease was obtained in the acidified series through the whole temperature range, while in the untreated set 80 to 85 per cent of grapefruit as well as sour-orange seedlings were killed by damping-off. Under these conditions no effect of soil temperature on the severity of the disease was apparent.

#### RELATION OF RESULTS TO BIOLOGICAL CONTROL IN GENERAL

It has been repeatedly reported that the growth of *Rhizoctonia* decreases at very acid reactions.<sup>(9, 22, 23, 24)</sup> Most of the observations have been based on measuring the diameter of cultures of *Rhizoctonia* in petri dishes. Since the fungus forms a much thinner web of hyphae in acid media than in neutral and alkaline ones, it seems probable that strong acidity diminishes the growth of the fungus even more than these data indicate. The preceding experiments, however, have shown that at the most acid reactions (pH 3.9 to 4.0) which were used, the fungus grows well enough to cause damping-off of citrus seedlings, if the sterilized soil is kept free from other organisms until the seed has germinated. The control of the disease so frequently obtained in nonsterilized acid soils without artificial introduction of other organisms may thus be due primarily to a change in the biological equilibrium of these soils caused by acidification.

This change seems to involve not only a decreased growth of *Rhizoctonia* but also an increase of the parasitic, antagonistic, and competitive activities of other organisms. *Trichoderma* spp. are reported to be very abundant in acid soils,<sup>(10, 20, 27)</sup> and under the conditions of our experiments they were found to become more active with increased acidity of the soil. The control that has been frequently obtained in combating the damping-off of pine seedlings, by acidifying the soil with aluminum sulfate or sulfuric acid,<sup>(10, 14, 22)</sup> may be due to the same factors.

A similar case has been reported with the take-all disease of wheat.<sup>(6, 7, 18, 24)</sup> The protective action of certain soils against this disease has been connected with biological factors, since sterilizing these soils destroys the biological control effect. Soil reaction does not seem to play a rôle here.

In the biological control of insect pests, the parasites are mostly obligatory, that is, they depend on the presence of hosts. The problem of biological control of soil-borne plant pathogens seems to be entirely different because the parasitic and antagonistic organisms thus far found are common soil saprophytes. There are reports on many such organisms other than *Trichoderma* that are able to act on *Rhizoctonia*.<sup>(6, 20)</sup> In the work presented here, control of the disease was not obtained by abundant inoculation of natural soils with the spores of *Trichoderma* if the soil reaction was not sufficiently acid. It seems, therefore, that primary emphasis has to be placed on creating favorable conditions for growth and antagonistic activity of the beneficial organisms, preferably conditions which also are unfavorable for the disease-inciting organism. In some cases, apparently, increase in control may be obtained by abundant inoculation with beneficial organisms, especially if the original soil flora is deficient in organisms effective under the new conditions. It is evident that fundamental studies under controlled conditions are necessary to gain a better understanding of the deciding factors in this problem. They may make it possible to develop methods of biological control and to select efficient antagonistic organisms.

### SEED-BED EXPERIMENTS

Some nurserymen believe that planting the citrus seed on ridges and irrigating in furrows will keep down damping-off, since by this method the surface of the soil is kept rather dry. Others find it more practical to broadcast the seed and to water by sprinkling. The latter method was followed in two field experiments conducted in 1934 at the Cascade Ranch, San Fernando, California.<sup>7</sup> According to the common practice,

<sup>7</sup> The authors are indebted to Mr. J. T. Culbertson for his splendid coöperation, which made this work possible.

the seed was covered by a layer of sand,  $\frac{5}{8}$  to  $\frac{3}{4}$  inch in depth. This sand is said to have the advantages of preventing the formation of a crust, of providing good aeration, and of keeping the surface rather dry after irrigating.

#### EXPERIMENT 1

The seed bed was divided into plots marked by the posts which supported the lath covering. The size of each plot was 85 square feet. The first experiment was started in the middle of April, a common time for sowing citrus seeds in this region. Twenty-three plots were planted to sweet orange, and 12 plots to sour-orange seed. The reactions to damping-off in the sweet and sour-orange plots were so similar under a given treatment that these are not separated in reporting the results. In an area that had been used during the previous year as a seed bed ("old" bed of table 1), there were 25 plots, 12 treated and 13 untreated, alternating with the others as checks. In another area of 10 plots of sour-orange seedlings, located some distance away, and not previously used as a seed bed ("new" bed), there were 6 treated and 4 check plots. This gave for the first experiment 6 plots for each of the following three treatments interspersed with 17 check plots.

*Formaldehyde Dust.*—A 6 per cent formaldehyde dust was mixed into the upper 3 inches of the soil 3 days before sowing, and the soil was then sprinkled lightly. The originators of this method<sup>10</sup> recommended  $1\frac{1}{2}$  ounces of dust per square foot mixed with the soil to a depth of  $2\frac{1}{2}$  to 3 inches. In several preliminary pot experiments, it had been found necessary to apply 3 ounces of formaldehyde dust per square foot in order to control heavy infections of *Rhizoctonia*. If used 2 or 3 days before sowing, this amount had no harmful effect on the germination of citrus seeds. It had been noted, however, that the treatment was not effective if the *Rhizoctonia* was introduced in the pots some time after sowing.

*German Peat.*—On the basis of the results of the greenhouse experiments reported above, the seed was planted between two layers of peat moss, each  $\frac{3}{4}$  inch deep. Simultaneously with the planting, the peat was inoculated with giant cultures of *Trichoderma* of three types: (1) pigmented cultures of *T. lignorum*, (2) "odorous" cultures of *T. lignorum*, and (3) *T. koningi*. The *Trichoderma* cultures had been grown for 1 week on peat which was saturated with a glycerine medium.

*Formaldehyde Dust and Peat.*—This is a combination of treatments 1 and 2. Only half the amount of formaldehyde was applied, and the seed was covered but not underlaid with peat moss.

*Results of Treatments in Experiment 1.*—The reaction of the soil,

which has the nature of a Hanford sandy loam, varied from pH 6.6 to 6.7, while that of the sand cover was from pH 7.6 to 7.9. These reactions remained practically the same in the check plots during the period of damping-off. In the peat moss, however, there was a change of the pH from 3.9-4.0 to 4.5-4.6 after 6 weeks and to 4.9-5.5 after 14 weeks. In all plots treated with peat about three-fifths of the area in which the seed-

TABLE 1

**EFFECT OF FORMALDEHYDE DUST AND PEAT SEED-BED TREATMENT ON RHIZOCTONIA DAMPING-OFF OF CITRUS SEEDLINGS IN EXPERIMENT 1**  
 (Planted April, 1934; results noted in October, 1934)

Treatment	Number of plots, 85 square feet each	Area affected with damping-off*		
		Total square inches	Square inches per plot	Per cent
New seed bed				
Check.....	4	6,399	1,600	13.0
Formaldehyde dust.....	2	2,731	1,365	11.1
Formaldehyde dust and peat cover.....	2	1,769	884	7.2
Peat.....	2	253	126	1.0
Old seed bed				
Check.....	13	11,507	885	7.2
Formaldehyde dust.....	4	8,680	2,170	17.7
Formaldehyde dust and peat cover.....	4	1,012	253	2.1
Peat.....	4	19	5	0.05

\* Damping-off rather uniformly distributed in new bed but very unevenly in old seed bed.

lings were attacked by the fungus showed the disease more than three and a half months after sowing. In the other plots practically all of the damping-off occurred in the first two or three months, most of it in the first 6 weeks. This coincidence of decrease in acidity of the peat with late damping-off is very suggestive, for it corresponds to our results in the experiments reported above.

As most of the damping-off developed in circular spots, the areas which were destroyed could be easily calculated from the diameters measured. Dead seedlings and attacked plants with dark-brown lesions at the stem base mark the margin of the areas. Table 1 represents the results six months after the planting of the seeds.

It should be emphasized that the areas affected with damping-off were rather evenly distributed over the 10 plots of sour-orange seedlings (new seed bed). In the other bed (old bed), most of the disease was in one section of the bed containing 2 formaldehyde-dust-treated plots. The series with the 10 plots gives, therefore, a truer picture of the actual situation.

## EXPERIMENT 2

A second experiment was started two months after the first. Thirteen plots adjacent to row 2 of the first experiment were planted with sweet orange, and, in addition, nearly 4 plots in the next row were planted with grapefruit and 1 with rough-lemon seed. Again each alternate plot was left untreated. The peat treatment had shown much promise in controll-

TABLE 2

EFFECT OF ALUMINUM SULFATE AND PEAT SEED-BED TREATMENT ON RHIZOCTONIA DAMPING-OFF OF CITRUS SEEDLINGS IN EXPERIMENT 2  
(Planted June, 1934; results noted in December, 1934)

Kind of seedlings	Treatment	Number of plots 85 square feet each	Area affected with damping-off*		
			Total square inches	Square inches per plot	Per cent
Sweet orange.....	Check.....	7	26,603	3,800	31.0
	Aluminum sulfate.....	2	417	208	1.7
	Aluminum sulfate and peat cover.....	2	716	358	2.9
	Peat.....	2	1,004	502	4.1
Grapefruit.....	Check.....	2½	12,097	4,400	35.9
	Aluminum sulfate and peat cover:.....	1	330	330	2.7
Rough lemon.....	Peat.....	1	237	237	1.9

\* Damping-off uniformly distributed.

ing the disease, so it was used again. As a second treatment, aluminum sulfate was applied at the rate of 35 grams per square foot. It was spread over the surface just before sowing, and raked into the top inch of the soil. The third treatment was a combination of the first two, the soil being treated with aluminum sulfate at the rate of 25 grams per square foot, and the seed covered but not underlaid with peat. The peat was inoculated with beneficial fungi as previously; to the soil treated with aluminum sulfate, fungi were not added.

*Results of Treatments in Experiment 2.*—The aluminum sulfate made the reaction of the upper soil inch much more acid (pH 3.5 to 3.6) than was expected from preliminary tests. The reaction of this soil was pH 4.0 after 6 weeks, and pH 4.7 to 4.9 after 4 months, while that of the peat became pH 5.0, and the soil below the peat had a reaction of pH 5.6. There was some delay of germination in plots treated with aluminum sulfate, but later the seedlings seemed to catch up with those in the check plots. The more frequent watering necessitated by the higher tempera-

tures after the late sowing may have contributed to the higher percentage of damping-off in the second experiment (table 2).

In the treated plots, the damping-off again developed later than in the checks, that is, two or three months after sowing. The very effective control of the disease obtained by the acidification treatments in the second experiment offset or at least masked any detrimental effect on the growth of the seedlings.

#### EFFECT OF ACIDIFICATION ON GROWTH OF SEEDLINGS

In the first experiment, there was a definite stunting effect discernible in the peat-treated plots. The average size, vigor, and stem diameter of the seedlings were evidently decreased in comparison with the adjoining check plots. It is thought that relatively lower soil temperature in and under the peat may be one of the factors that contribute to a slower growth of the seedlings, in addition to the temporary effect of excessive acidity on the root development of citrus seedlings, the unfavorable influence of which has been reported.<sup>10</sup>

There had been previously observed in greenhouse experiments certain detrimental effects of peat on sweet-orange seedlings. The roots of some seedlings developed poorly or were stunted. It had been expected that in the field the plants would reach the less acid subsoil soon and would overcome any detrimental effect easily. In fact the contrary was true, perhaps because the frequent watering in the greenhouse brought about a much more rapid decrease of acidity in the peat than would appear in the field.

An attempt was made to remedy the stunting effect of the peat. In addition to the fertilization of the checks with dried blood and calcium nitrate, when the seedlings were six months old the following three corrective treatments were tried in halves of the treated plots, the other halves receiving calcium nitrate only: (1) 11 pounds of quicklime per 100 square feet; or (2) 1 pound of bordeaux powder per 100 square feet; or (3) 2 pounds of a zinc sulfate and lime powder containing equal parts of lime and  $ZnSO_4$  per 100 square feet. None of these treatments showed any considerable improvement over the checks.

Thus, in spite of the good control of damping-off obtained, the acidifying methods employed here, especially the peat treatment, cannot be recommended yet for general use on account of the stunting effect on the seedlings. The most promising of the treatments so far seems to be the aluminum sulfate.<sup>9</sup> It is more adaptable to modification than the peat

<sup>9</sup> As this goes to press the results of 1935 experiments with 30 grams of aluminum sulfate per square foot have shown only 2 per cent damping-off against 39 per cent in adjacent checks, and no stunting effect on the seedlings.

treatment, which is inconvenient to apply and seems to keep the soil too cool for the best root growth. The value of adding beneficial fungi with the acidifying treatments remains to be seen through future investigation. Increased control was obtained in some greenhouse experiments. No comparable experiments were made in the field.

### SUMMARY

Damping-off of citrus seedlings, caused by *Rhizoctonia solani*, was successfully controlled in laboratory, greenhouse, and field experiments by acidifying the soil layers next to the seed by the application of aluminum sulfate or acid peat moss, which produced an initial reaction of about pH 4.0.

However, damping-off was not controlled in sterilized soils of the same acidity in the absence of *Trichoderma* spp. Therefore, the control of the disease by the acidification of nonsterile soil cannot be explained entirely on the grounds that such a medium is unfavorable to the growth of *Rhizoctonia*. Evidence is given which indicates that the decisive factor is a change in the microflora of the soil, favoring organisms such as *Trichoderma*, which may be antagonistic or parasitic towards *Rhizoctonia solani*.

In some cases, with moderately acidified natural soils, abundant inoculation with *Trichoderma* spp. was accompanied by a larger percentage of healthy seedlings. Such biological control was entirely absent in soil of neutral reactions.

Growing seedlings at a series of constant soil temperatures from 18° to 35° C seemed to affect neither the severity of damping-off due to *Rhizoctonia solani* nor the degree of control of the disease through applying aluminum sulfate.

The addition of peat moss to the seed bed proved unsatisfactory from the commercial standpoint, since it had a stunting effect on most of the seedlings. Treatment with aluminum sulfate appears promising as a practical method for controlling the damping-off disease of citrus seedlings.

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INHERITANCE OF RESISTANCE TO BUNT,  
TILLETIA TRITICI, IN HYBRIDS OF  
TURKEY WHEATS C. I. 1558B  
AND C. I. 2578

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FRED N. BRIGGS<sup>3</sup>

INTRODUCTION

TURKEY is the name most commonly applied to the Crimean group of hard red winter wheats grown in the United States. In 1924, according to Clark and his co-workers,<sup>(1)</sup> the hard red winter wheats comprised 41.4 per cent of the total wheat acreage in this country; and Turkey, including Kanred, made up 91.7 per cent of the acreage devoted to hard red winter wheat. At that time, therefore, over 36 per cent of the entire wheat acreage was devoted to Turkey. This type of wheat was first brought to the United States in 1873 and was grown in Kansas.<sup>(2)</sup> Since that time numerous introductions have been made both by private and by public agencies. Other names that have been applied to the type are Alberta Red, Argentine, Bulgarian, Crimean, Defiance, Egyptian, Hard Winter, Hundred-and-One, Hungarian, Improved Turkey, Kharkoff, Lost Freight, Malecome, Malakof, Minnesota Red Cross, Minnesota Reliable, Pioneer Turkey, Red Russian, Red Winter, Romanella, Russian, Taurenian, Theiss, Turkey Red, Turkish Red, Ulta, Wisconsin No. 18, and World's Champion.

Recently certain strains of Turkey wheat have been distributed under other varietal names based on performance records and slight morphological differences. It has been long recognized that there are both morphological and physiological differences between certain of these Turkey strains. Sherman<sup>(3)</sup> and Oro<sup>(4)</sup> are two such wheats.

The Turkey wheats have been an important source of varieties resistant to bunt (*Tilletia tritici*), for genetic studies and for the production of other resistant varieties. Of the 17 most resistant varieties discovered by Tisdale and his co-workers,<sup>(5)</sup> 11 were Turkey wheats. Two others, Banner Berkeley and Ridit, resulted from hybrids that had Turkey for

<sup>1</sup> Received for publication July 6, 1935.

<sup>2</sup> The writer wishes to acknowledge the assistance of S. B. Akins and G. L. Barry in making smut counts.

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one parent. Kiesselbach and Anderson<sup>(12)</sup> isolated 12 resistant lines from Turkey (South Dakota 144) wheat. Other lines showed varying amounts of smut up to more than 90 per cent.

The genetics of bunt resistance has been studied by the present writer<sup>(7)</sup> in 9 resistant varieties of wheat. Four of these—Turkey 1558, Turkey 3055, Sherman, and Oro—have been Turkey types. Two of the three genetic factors found are represented in these wheats. Sherman has the Martin factor,<sup>(7)</sup> whereas Oro, Turkey 1558, and Turkey 3055 have the Turkey factor.<sup>(7)</sup>

TABLE 1

ANNUAL PERCENTAGES OF BUNT INFECTION AT DAVIS, CALIFORNIA, IN THE PARENT WHEAT VARIETIES DURING THE YEARS INDICATED

Parent variety	Percentage of bunted plants						
	1929	1930	1931	1932	1933	1934	Average
Turkey 1558B.....	0.0	1.1	0.0	1.9	0.0	0.8	0.6
Turkey 2578.....	0.0	2.9	1.9	0.0	0.0	1.3	1.0
Turkey 3055.....	0.1	2.0	1.2	1.8	0.1	0.3	0.9
Martin.....	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Selection 1403.....	....	0.0	0.0	0.0	0.0	1.4	0.3
Baart.....	....	....	47.2	85.5	66.0	84.4	70.8
White Federation.....	78.6	59.3	43.0	73.2	59.9	73.7	64.7

The inheritance of resistance to bunt has been studied in hybrids involving two other resistant varieties of the Turkey type—namely, Turkey 1558B and Turkey 2578. The former is from a pure-line selection made at Moro, Oregon, by Carleton R. Ball. The history of Turkey 2578 is unknown to the present writer.

### EXPERIMENTAL RESULTS

The parental material and hybrid populations were grown in the field at the University Farm, Davis, California. The methods of handling and the inoculum used have been described in previous publications.<sup>(2-6)</sup> The collection of bunt has been designated as physiologic race III of *Tilletia tritici* by Reed<sup>(13)</sup> and physiologic form VIII by Bressman.<sup>(14)</sup>

The percentage of bunt infection in the parent varieties may be seen in table 1.

The percentage of bunted plants in each case is based on 2 or more rod rows. Usually 30 or more rows of the susceptible parent were grown, and frequently 10 or more rows of the resistant parent were included. There were 30 to 70 plants per row. The difference between resistant and susceptible parents is very marked.

Turkey 1558B and Turkey 2578 were crossed with Baart to determine

the number of bunt-resistant factors in each of these two resistant varieties. Crosses using these were also made with Martin, Turkey 3055, and Selection 1403 to test for the presence of the Martin, Turkey, and Hussar factor respectively. The last-named cross was grown only in  $F_2$ .

$F_1$  seeds were not inoculated, because of the small number available. Where  $F_2$  plants were being grown as a source of seed to be inoculated in  $F_3$ , they were kept free from bunt in order to prevent the elimination of susceptible progeny by bunt in  $F_2$ .

TABLE 2

PERCENTAGE OF BUNTED PLANTS IN THE PARENTS AND IN  $F_2$  OF THE CROSSES NAMED;  
DAVIS, CALIFORNIA, 1934

Parent or cross	Number of plants grown	Number of plants bunted	Per cent of plants bunted
Turkey 1558B.....	1,276	12	0.9
Baart.....	2,155	1,822	84.5
Turkey 2578.....	1,197	17	1.4
Martin.....	432	0	0.0
Selection 1403.....	345	5	1.4
Turkey.....	391	1	0.3
Turkey 1558B $\times$ Baart.....	711	236	33.2
Turkey 1558B $\times$ Martin.....	1,066	71	6.7
Turkey 1558B $\times$ Turkey 3055.....	389	4	1.0
Selection 1403 $\times$ Turkey 1558B.....	481	61	12.7
Turkey 2578 $\times$ Baart.....	827	310	37.5
Turkey 2578 $\times$ Martin.....	825	33	4.0
Turkey 2578 $\times$ Turkey 3055.....	811	27	3.3
Selection 1403 $\times$ Turkey 2578.....	501	48	9.6

$F_2$  populations of all the crosses were inoculated and grown in 1934 along with the  $F_3$ . Although  $F_2$  data do not permit a complete Mendelian analysis, they do indicate the number of resistant factors present as well as their identity and effect.

The  $F_2$  data, accordingly, are also included in table 2.

The classification of the  $F_2$  plants on the basis of the bunt obtained in  $F_3$  rows gives much more satisfactory data. These rows contained from 30 to 70 plants, usually about 50. In most cases this classification is certain and reliable. Concerning a few rows that fall near the minima there is some uncertainty; but these rows are relatively few (table 3).

The rows in the 0-5 per cent class for bunt infection were subdivided into those without any bunt and those with 1-5 per cent.

The hybrids with Turkey 1558B may be considered first. The distribution of rows in Turkey 1558B  $\times$  Baart is shown in figure 1. The number of rows under the three modes is very near the 1:2:1 ratio. Accepting 12.5 and 47.5 as minima, there are 60 resistant, 123 segregating, and 62 sus-

TABLE 3  
DISTRIBUTION OF PARENT AND F<sub>1</sub> ROWS OF THE CROSSES NAMED INTO 5 PER CENT CLASSES FOR BUNT INFECTION;  
DAVIS, CALIFORNIA, 1934

Parent or cross	Distribution of rows having a bunt infection (per cent) of —															Total number of rows				
	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	70-75	75-80	80-85	85-90	90-95	95-100
Turkey 1558B.....	8	8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	16
Turkey 2578.....	7	9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	16
Baart.....	8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	42
Martin.....	7	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	8
Turkey 3055.....	7	29	18	12	13	27	30	21	18	6	4	7	6	10	5	12	8	6	3	245
Turkey 1558B × Baart.....	52	40	16	3	1	1	1	1	1	0	1	2	0	0	0	1	1	1	1	113
Turkey 1558B × Turkey 3055.....	50	23	16	8	7	5	3	1	1	0	1	2	0	0	0	1	1	1	1	118
Martin × Turkey 1558B.....	14	21	22	8	11	20	20	30	18	9	11	10	5	14	8	6	7	2	2	255
Turkey 2578 × Turkey 3055.....	69	37	10	3	2	1	2	0	1	0	3	0	1	1	0	0	1	1	1	121
Martin × Turkey 2578.....	48	24	21	16	1	2	0	1	0	3	0	1	1	0	0	1	1	1	1	119

ceptible rows where 61.25, 122.5, and 61.25 are the numbers expected. Turkey 1558B therefore differs from Baart in one major factor for resistance to bunt. Both  $F_2$  and  $F_3$  data indicate that resistance is incompletely dominant.

The identity of the factor for resistance to bunt in Turkey 1558B is established by the cross with Turkey 3055, the tester for the Turkey factor. No susceptible rows occurred in a population of 113 rows which

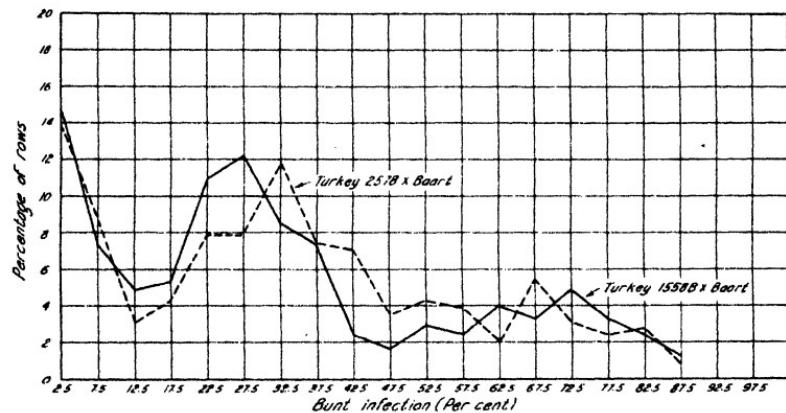


Fig. 1.—Distribution of  $F_3$  rows of Turkey 1558B  $\times$  Baart and Turkey 2578  $\times$  Baart into 5 per cent classes of bunt infection.

shows that Turkey 1558B is identical with Turkey 3055 as regards its major factor for resistance to bunt and therefore has the Turkey factor for resistance.

The hybrids with Turkey 2578 may now be considered briefly. The distribution of rows in Turkey 2578  $\times$  Baart (fig. 1) resembles that of Turkey 1558B  $\times$  Baart. If we accept 12.5 and 47.5 as minima, the agreement with the 1:2:1 ratio is very close. There is not a very clear-cut minimum at 47.5. In practically all other crosses between resistant and susceptible varieties, however, there has been a fairly well-defined minimum in this region. If the susceptible and segregating rows are added together, a very good 3:1 ratio is obtained. Further evidence that the resistance of Turkey 2578 results from a single factor is furnished by the cross with Martin. There are 3 susceptible rows in a population of 119—a satisfactory agreement with the 15:1 ratio. The value of  $P$  is between 0.1 and 0.2. The segregation is similar to that obtained in the cross of Martin  $\times$  Turkey 1558B and in other crosses where the Martin and Turkey factors were present. In view of these considerations, the

data indicate that Turkey 2578 differs from Baart in one major factor for resistance to bunt. Here again, resistance is incompletely dominant.

The identity of the factor for resistance to bunt in Turkey 2578 is established by the cross with Turkey 3055. There were no susceptible rows in a population of 121 rows.

#### DISCUSSION AND SUMMARY

Both Turkey 1558B and Turkey 2578 were found to depend on the Turkey factor only for their resistance to bunt. This now makes a total of five varieties found to have the Turkey factor.<sup>(7)</sup> All are of the Turkey type of wheat.

One variety, Sherman, of the Turkey type has been found to have the Martin factor for resistance. Thus two of the three major factors discovered by the writer<sup>(7)</sup> are known to be present in Turkey wheats. It should be relatively easy, accordingly, to combine these into a single variety of the Turkey type.

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## NITROGEN TRICHLORIDE AND OTHER GASES AS FUNGICIDES<sup>1, 2, 3</sup>

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### INTRODUCTION

OBVIOUS ADVANTAGES are to be had in the use of a suitable gas for the control of fungi and insect pests on fruits in storage rooms and in cars during shipment. Application would be relatively simple and inexpensive; the material, because of its fluidity and diffusibility would, if aided by agitators, penetrate quickly to all exposed surfaces; and at the termination of the treatment, the gas could be readily eliminated by forced ventilation. Where tight refrigerator cars are used, it is possible that the fruit might remain in a low but effective concentration of the protective gas during shipment, assuring minimum losses from decay.

Nitrogen trichloride, the gas with which this report is chiefly concerned, is used extensively in the treatment of freshly milled flour in order to mature it quickly and induce desirable baking qualities. The suggestion that the gas might be used for the control of citrus pests was made several years ago when the Field Department Laboratory of the California Fruit Growers' Exchange and the Wallace and Tiernan Products Company began a series of coöperative experiments. At that time tests were made to determine whether or not the gas would decrease losses from the most serious organisms of decay in citrus fruits, namely, blue and green molds, or *Penicillium italicum* and *P. digitatum*. Such promis-

<sup>1</sup> Received for publication August 15, 1935.

<sup>2</sup> Paper No. 321, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

<sup>3</sup> The results of this paper grew out of coöperative investigations with the staff of the Field Department Laboratory of the California Fruit Growers' Exchange and of the Wallace and Tiernan Products Company, Inc. To the staff of the former under the direction of A. E. Nelson, coöoperating with J. C. Baker and T. E. Galvin of the latter, the author is indebted for facilities and data acknowledged later.

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ing experimental results with small lots of fruit were secured that trials with commercial carload lots and larger were made. The method has recently been adapted to the protection of fruit in storage rooms and refrigerator cars, and is now being used commercially by fifteen packing companies.

### REVIEW OF PREVIOUS INVESTIGATIONS

A review of the literature reveals that several investigators have thought of the possibilities of gases as fungicides. Ludwig<sup>(7, 8)</sup> grew a number of bacteria and fungi in several concentrations of illuminating gas, finding the organisms relatively insensitive to concentrations even as great as 25 per cent of gas in air. Studying the individual components of the illuminating gas, he found the toxicity of carbon monoxide about equal to, and that of methane and ethylene less than, that of the complete gas. Ethylene and methane showed scarcely any toxicity below a concentration of 40 per cent.

Oserkowsky<sup>(10)</sup> found that at 30° C (86° F) the hyphae of *Sclerotium rolfsii* were killed by 2 to 4 days' exposure to air saturated with the vapors of any one of the following: trioxy-methylene, benzene, toluene, xylene, nitrobenzene, and the ortho, meta, and para forms of dichlorobenzene.

Neifert and Garrison<sup>(20)</sup> showed that cyanogen chloride in concentrations of 3 per cent or greater for 2 hours was effective in killing species of *Fusarium*, *Ascochyta*, *Penicillium*, *Colletotrichum*, and *Sclerotium*.

Brooks *et al.*<sup>(9)</sup> report that a concentration of 37 per cent carbon dioxide in air completely inhibited the growth of *Botrytis cinerea* and *Rhizopus nigricans* and decay of strawberries by these fungi. Thirty per cent or more of the gas prevented decay of prunes by *Sclerotinia fructicola* and of peaches by *B. cinerea*, and greatly inhibited decay of peaches due to *S. fructicola* and *R. nigricans*.

Brown<sup>(10)</sup> showed carbon dioxide to be more inhibitive to spore germination than to hyphal growth, and to be more effective in retarding growth in water than in nutrient media, and at low rather than at high temperatures. He suggested a concentration of 10 to 20 per cent to reduce decay of apples in cold storage. Kidd, West, and Kidd<sup>(10)</sup> determined that an atmosphere containing 10 per cent carbon dioxide and 11 per cent oxygen was most suitable for apple storage.

Barker<sup>(10)</sup> found that after 5 weeks at 1.1° or 4.9° C (34° or 41° F), 50 per cent carbon dioxide produced severe injury in African Navel orange, and that the injuries were aggravated by low oxygen content. On the other hand, where the oxygen content was sufficient for respira-

tion, high concentrations of carbon dioxide at 7.1° to 12.6° C (45° to 55° F) did not result in injury during the same period of storage. Thornton<sup>(13)</sup> states, moreover, that the Foster Pink and Thompson varieties of grapefruit and Valencia oranges at a temperature range of 0° to 21° C (32° to 69.8° F) showed no injury after 7 days' exposure to 50 per cent carbon dioxide, but were injured by 65 per cent carbon dioxide; that the Walters grapefruit, King oranges, and tangerines withstood 25 per cent but were injured by 50 per cent of the gas.

Winkler and Jacob<sup>(15)</sup> and Jacob<sup>(16)</sup> found that for grapes an absorption of 50 to 100 parts of sulfur dioxide per million parts of the fruit retarded the rate of deterioration about one-half. Pentzer, Asbury, and Hamner,<sup>(18, 19)</sup> studying the rate of absorption of sulfur dioxide by several varieties of grapes, found that not more than 20 parts per million should be used for the varieties grown in the San Joaquin Valley. Pentzer and Asbury<sup>(19)</sup> showed that for a period of 12 days, 5 parts per million in Sultaniña grapes at 11.7° C (53° F) reduced decay from 20 per cent to less than 1 per cent; 46 parts per million gave complete protection but caused slight injury. Utilization of the sulfur dioxide slowly released by acid sodium sulfite in pads or sawdust packs showed promise.

Brief mention is here made of two papers on the use of formaldehyde vapor. To prevent potato scab, Morse<sup>(20)</sup> treated seed potatoes with formaldehyde gas, using per 1,000 cubic feet of space (10 pounds of tubers per cubic foot) 3 pints of formalin with 23 ounces of potassium permanganate for vaporization. Thomas,<sup>(21)</sup> in his work on the disinfection of seeds with formaldehyde vapor, was able to kill *Monilia fructigena*, *Ascochyta* sp., *Colletotrichum gloeosporioides*, and *Bacillus carotovorus* by steam vaporization of 20 ounces of formalin per 1,000 cubic feet for 2 hours. Four species of *Fusarium* survived the treatment.

Preliminary work by Trout and Tomkins<sup>(22)</sup> indicates that the concentrations of acetaldehyde which will protect oranges from decay also cause injury to the rind of the fruit. With grapes, continuous storage in a concentration of 1/1,000 acetaldehyde markedly retarded the development of molds without apparent chemical injury. Short-period exposures (34 to 96 hours) to a 1/250 concentration at 25° C (77° F) were unsatisfactory, the shorter exposures not sufficiently checking mold growth and the longer exposures causing the development of an aldehyde taste in the fruit. At 1° C (33.8° F), however, grapes exposed 72 hours to a 1/250 concentration of the gaseous aldehyde were kept in good condition for 36 hours. The method also showed promise with cherries and prunes.

The same authors, Tomkins and Trout,<sup>(22)</sup> using ammonia solutions in

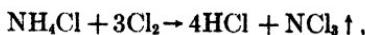
a system similar to that used for acetaldehyde, found that at 18° C (64.4° F) the introduction of volume concentrations of 5 to 10 parts per 10,000 lessened decay in wounded oranges inoculated with *Penicillium digitatum*. At lower temperatures, such as 10° C (50° F) and 3° C (37.4° F), volume concentrations as low as 1 part in 10,000 were effective. However, the lower temperatures also rendered the fruit more susceptible to ammonia injury. At 18° C (64.4° F) the rind would tolerate 10 parts per 10,000, but at 3° C (37.4° F) was injured at the wounds by a concentration of 3 parts per 10,000. Crystals of ammonium carbonate and ammonium bicarbonate were also found to supply a concentration of ammonia sufficient to lessen decay due to green mold, provided the conditions of storage were such that the crystals kept moist.

Tomkins<sup>(19)</sup> exposed agar cultures of *Trichoderma lignorum*, *Rhizopus nigricans*, *Thielaviopsis paradoxa*, *Gloeosporium musarum*, and *Botrytis cinerea* to several vapors and gases and compared the effects on germination and growth with the behavior of the fungi in air. The reactions of the several fungi were similar, and he confines the details of the report to *Trichoderma lignorum*. At 25° C (77° F) and in a closed container of 800-cc capacity, germination and growth of *T. lignorum* were greatly inhibited by quantities "greater than" 0.144 cc of hydrogen sulfide and 224 cc of hydrocyanic acid gas, and by the vapor from the following volumes of materials in respective 400 cc quantities of aqueous solutions: 0.2 cc chloroform, 2.4 cc acetal, 1.4 cc ethyl ether, 0.6 cc amyl formate, 12 cc ethyl alcohol, and 0.04 cc of concentrated ammonia solution. Germination was also inhibited by the vapor from 14 cc of acetone or 0.1 cc of acetaldehyde.

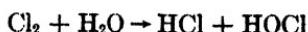
Wilcoxon and McCallan<sup>(20)</sup> found hydrogen sulfide very toxic to the conidia of *Sclerotinia americana*.

#### MANUFACTURE AND USE OF NITROGEN TRICHLORIDE

Chlorine and ammonium chloride are the materials employed in the generation of nitrogen trichloride. Empirically, the equation for the formation of the chemical may be written:



but according to J. C. Baker,<sup>\*</sup> who developed the method, the reaction probably proceeds in two steps, the hypochlorous acid formed by the reaction of chlorine and water reacting at once with the ammonium salt:




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\* Personal interview, September 28, 1931.

Since gaseous  $\text{NCl}_3$  is 4.18 times as heavy as air and readily collects in explosive droplets, it must be handled in dilution and cannot be accumulated in quantity and stored in metal cylinders for future use. To be handled safely it must be prepared with special apparatus which generates it as a gas highly diluted with air. The low concentrations effective

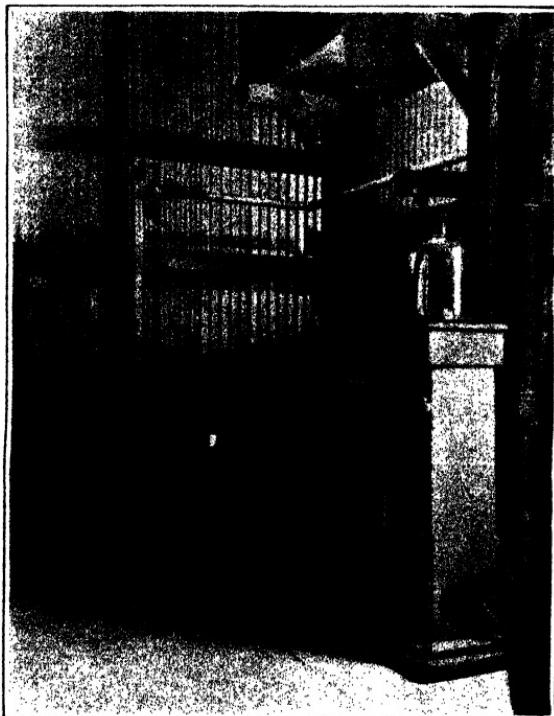


Fig. 1.—Single-stream  $\text{NCl}_3$  generating unit used in experimental work.

for mold control are so irritating to the eyes and mucous membranes that the gas thus gives adequate warning before serious injury to humans can occur. With the equipment shown in figures 1 and 2, known quantities of the gas can be metered with known volumes of air; thus the concentration can be well controlled. However, for the safe and effective treatment of citrus fruits a trained and constantly attentive operator must be employed.

As applied to citrus fruits, the gas-air mixture is introduced into a refrigerator car or storage room and made to circulate rapidly by the use of electric fans. Determinations of the residual gas content of the

room are made from time to time by the use of a simple, rapid colorimetric method with orthotolidine as the indicator. By residual is here meant the concentration of the gas remaining in the air after the contents and walls of a room are saturated.

The procedure chosen in applying the gas treatment depends upon the manner of handling the fruit at the packing-house. There are three

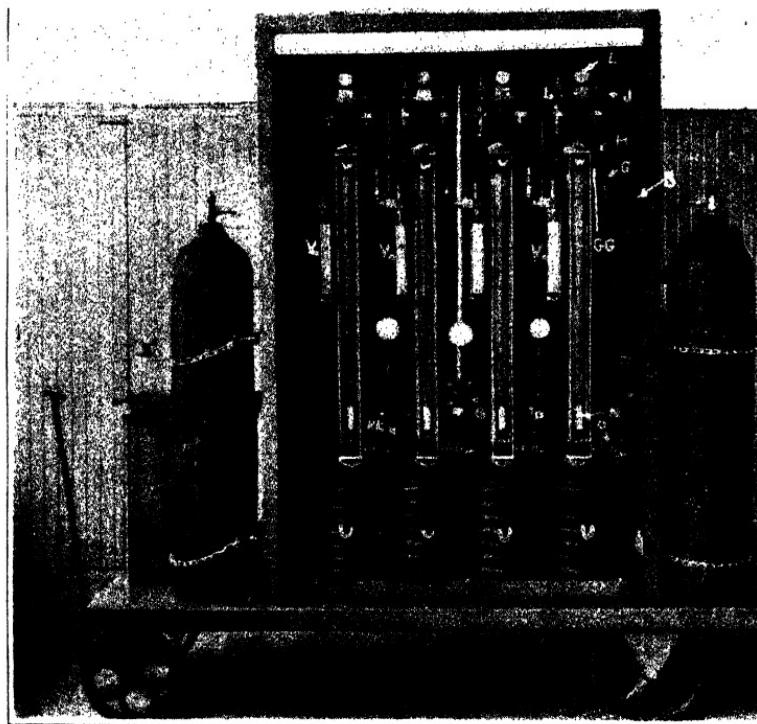


Fig. 2.—Four-stream NCl<sub>3</sub> generating unit used in citrus industry.

general procedures: namely, long storage, short storage, and no storage or the immediate packing-out of the fruit. In long storages a sterilizing treatment of from 1 to 3 applications of gas (8 mg  $\text{NCl}_3$  per cubic foot) at intervals of 3 to 4 days is given when the fruit is first placed in the storage room. Additional gassings are made at weekly intervals to prevent reinfection. Just before removal of the fruit from storage another exposure is made to kill the fungus spores on any decay that may have developed, and to sterilize the field boxes in which the fruit was stored. This reduces the incidence of infection during the packing-house handling. Finally, the packed fruit is gassed in the refrigerator car to steril-

ize any injuries that were made during the packing and loading operations and to protect the fruit in transit.

For fruit that is held in storage only a short time the same procedure is followed as for long-storage fruit. After the first sterilizing treatment, however, there would necessarily be fewer gassings during the shorter storage period.

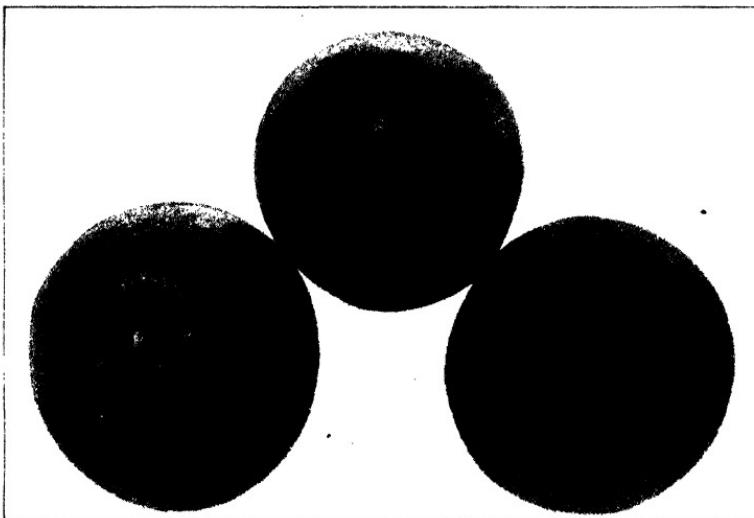


Fig. 3.—Wounded Washington Navel oranges showing collapse and browning of wounds after three 6-hour experimental treatments with high concentration (40 mg per cubic foot) of  $\text{NCl}_3$ . Such a high concentration is not used commercially.

Fruit that is not placed in the storage room, but is packed out and loaded at once, receives only the single gassing in the refrigerator car.

It should be noted here that the treatment with nitrogen trichloride may cause the rind tissues surrounding wounds to collapse and turn brown. The extent of such effect depends upon the concentration of and the duration of exposure to the gas. Figure 3 shows the result where an excessively high concentration of the gas was used. That such treated tissues offer unfavorable foci for decay was apparent early in the investigation.

#### EXPERIMENTS ON DECAY PREVENTION

*Tests with Small Lots of Fruit.*—Several experiments were conducted to determine the relative effectiveness of varying numbers of applications of gas in decreasing losses from decay due to *Penicillium italicum* and *P. digitatum*. Representative results are given in table 1. In the lots

called "injured and inoculated," Valencia oranges were wounded uniformly by a nail scratch on the shoulder before inoculation and treatment; in the lots marked "orchard run" the fruit received no wounds

TABLE 1

EFFECT OF NUMBER OF APPLICATIONS OF NITROGEN TRICHLORIDE IN CHECKING DECAY OF VALENCIA ORANGES DUE TO BLUE AND GREEN MOLDS, 1933  
(226 fruits used in each lot)

Lot No.	Pretreatment	Gassing*		Percentage of decay after			
		Number of times	Intervals, days	2 weeks	4 weeks	6 weeks	10 weeks
First series; treatment begun September 22							
1 2 3	Injured and inoculated	0 (Soap check)	..	96.9	98.7	100.0	....
		1	..	23.9	41.6	57.1	....
		9	4	15.5	19.9	21.2	....
4 5 6 7† 8	Orchard run, inoculated	0 (Soap check)	..	....	40.7	....	71.2
		1	..	....	8.4	....	23.4
		3	4	....	7.5	....	14.2
		3	4	....	4.9	....	14.6
		16‡	4	....	0.0	....	21.2
Second series; treatment begun October 5							
9 10 11 12 13 14 15 16 17 18 19 20 21 22	Injured and inoculated	0 (Soap check)	..	86.7	....	99.5	....
		0 (Soap check)	..	90.3	....	99.5	....
		1	..	13.7	....	29.6	....
		2	2	9.7	....	25.2	....
		2	3	11.1	....	26.1	....
		2	6	15.9	....	26.6	....
		3	6	15.9	....	25.2	....
		5	3	7.1	....	12.8	....
		6	1	10.2	....	18.1	....
		7	6	....	....	34.1	....
		8	2	9.7	....	17.7	....
		11§	1	....	....	18.6	....
		18	3	....	....	18.8	....
		16¶	2	....	....	14.2	....

\* Concentration of gas: 27-50 mg NCls per cubic foot. Exposure: 2-6 hours.

† Repetition of No. 6.

‡ Gassings on September 22, 26, 30, then at 4-day intervals from October 9.

§ Gassings at 1-day intervals October 5 to 10, then October 17, 25, 31, and November 6 and 14.

¶ Gassings at 2-day intervals October 5 to 21, then on October 25, 28, 31, and November 3, 6, 9, and 14.

prior to inoculation in addition to those already received in the orchard and in subsequent handling. Inoculation consisted of a single, quick "in and out" immersion of the fruit in a heavy spore suspension of the two molds.

Since the use of warm soap solutions to clean the fruits and lessen decay is a regular packing-house procedure, soap checks were used in

the experiments reported in table 1 for comparison with the gas treatments. "Soap check" means immersion for 1½ minutes in a ½ per cent soap solution at 46° C (115° F).

The results show that all the gas treatments of the oranges markedly lessened the decay. Lot 8, which had no visible decay at the end of 4 weeks, had received 7 gassings during that period, while lots 6 and 7, which had a decay of 7.5 per cent and 4.9 per cent respectively, had received but 3 gassings. At the end of 10 weeks, however, lot 8, which had then been given a total of 16 gassings, had 21.2 per cent decay, and lots 6 and 7, which had received no additional treatments, had only 14.2 per cent and 14.6 per cent respectively. This indicates that beyond a certain number of exposures fruit may not only receive no benefit from additional exposures, but may be actually adversely affected. Lot 16, which received but 5 gassings, developed less decay than lots that received more or fewer treatments. Early in the investigations, it was discovered that fruits which had received a large number of apparently noninjurious dosages of  $\text{NCl}_3$  acquired an aged, shriveled appearance, indicating a loss of vitality and of resistance to organisms of decay.

*Tests With Large Commercial Lots of Fruit.*--To determine the protective effect of gas treatments on Washington Navel oranges under actual commercial storage conditions and in transit in refrigerator cars, coöperative experiments were conducted in Tulare County during the Washington Navel orange season of 1933-34. Since the results of these investigations have been reported by Nelson and Nedvidek,<sup>11</sup> only a brief reference is made to them here. The results showed the marked protective effect of the gas treatments. At the end of a month's storage the untreated fruit from an orchard that produced relatively weak, decay-susceptible oranges had 16.44 per cent decay, while the gassed fruit from the same orchard had 4.31 per cent decay. Three weeks after packing, the untreated lot of fruit had 28.2 per cent and the treated had 5.6 per cent additional fruits decayed. The average losses for the lots of fruit from 13 different orchards were 4.16 per cent for the untreated and 1.06 per cent for the treated fruit at the end of the storage period, and 16.3 per cent and 5.2 per cent respectively 3 weeks after packing.

On arrival in New York, 11 days after packing, 9 boxes of the gas-treated fruit and 9 of the check fruit were, through coöperative arrangement, examined by C. O. Bratley, of the United States Department of Agriculture. In addition, 2 boxes of freshly picked fruit (not stored) that had been included in the gassed car and 2 boxes of fresh unstored fruit included in the untreated car were also examined. Fruit from the

storage rooms showed 0.6 per cent decay in the check boxes and 0.3 per cent in the gassed boxes. After being held 10 days at 18.3° C (65° F) and 65 per cent relative humidity, the checks had 8.5 per cent decay and the gas-treated fruit 1.2 per cent decay. The check boxes of fresh fruit from the car that had received no gas showed 3.7 per cent decay on arrival and 30.3 per cent 10 days later, while similar fruit that had received the one gas treatment had 1.6 per cent on arrival and 10.9 per cent 10 days later.

The summarized conclusions of a large number of experiments on the  $\text{NCl}_3$  treatment of oranges conducted as a coöperative project of the Field Department Laboratory of the California Fruit Growers' Exchange, the Citrus Experiment Station, and the Wallace and Tiernan Products Company, are given by Ramsey<sup>(10)</sup> in a circular issued by the California Fruit Growers' Exchange. Since this circular is not available to the entire citrus industry, some of the conclusions are given here.

1. When blue contact mold is not as prevalent as common green mold, and the fruit is held in the packing-house for only 2 or 3 days before packing, treatment of the oranges with a solution of borax and boric acid under the approved commercial methods will generally retard the decay to as great an extent as the  $\text{NCl}_3$  gas. If blue contact mold is prevalent, or the fruit is held for more than 3 days before packing and is gassed with the  $\text{NCl}_3$  gas at the proper times, the  $\text{NCl}_3$  gas treatment should show better results than a solution of borax and boric acid in reducing decay. The results secured with the gas have been obtained on experimental and semicommercial tests, and definite conclusions cannot be drawn until the process has been used for a year or two in some packing-house operating in the regular commercial manner.

2. Nitrogen trichloride gas in concentrations of 5 mg per cubic foot and upwards is lethal to the spores and mycelium of blue and green mold (*Penicillium italicum* and *P. digitatum*) and of various other fungi, such as *Alternaria citri*, *Colletotrichum gloeosporioides*, *Phytophthora citrophthora*, *Botryosphaeria ribis*, and *Oospora citri-aurantii*, the length of exposure to the gas being inversely related to the concentration of the gas.

3. The gas will penetrate the wrappers of a packed box of oranges loaded in a car and kill a large percentage of the mold spores on the fruit, provided there is sufficient air circulation. The use of two pre-cooling fans in a car will give sufficient air circulation.

4. With fruit shipped soon after picking, best results with the  $\text{NCl}_3$  gas can be obtained by treating the oranges as soon as possible after they are picked, again the night before packing, and then after the packed

fruit is loaded in the car. The gas will kill the spores on the surface of an orange which has already decayed but cannot reach the mycelium within the rind. Gassing the fruit before decay starts is therefore essential.

5. When oranges have been held in coloring rooms or air-conditioned rooms before packing and decay has developed, a treatment with gas will kill most of the spores. Such a precautionary measure will reduce infection of sound fruit during washing and packing operations.

6. Because the  $\text{NCl}_3$  gas is fatal to the spores whether they are on the fruit, on boxes, or in the air, continued use of the gas on all of the fruit in a packing-house should very materially reduce the spore load and thus reduce the chances of infecting sound fruit.

7. Too high a concentration of the gas or inadequate air circulation while it is being applied has resulted in a browning of injuries already on the fruit. This browning, which resembles pitting, can be readily avoided by adequate air circulation and an adequate control of the gas concentration by occasional tests and adjustments.

8. The  $\text{NCl}_3$  gas has a tendency with most lots of fruit to retard color development, but this has generally been overcome by applying slightly higher doses of ethylene or by increasing the time of coloring 12 to 24 hours.

#### EFFECT OF NITROGEN TRICHLORIDE AND OTHER GASES ON CITRUS FUNGI

The following experiments accompanied those reported on decay prevention and were planned to determine the dosages of  $\text{NCl}_3$  gas lethal to the several decay-producing organisms. Mention is made also of some experiments with chlorine, ozone, methylchloramines, and sulfur dioxide.

*Comparison of Nitrogen Trichloride and Chlorine.*--While more toxic to humans and consequently more dangerous to work in than  $\text{NCl}_3$  gas, chlorine gas has the advantages of being nonexplosive and hence obtainable in metal cylinders which would permit easy application. Accordingly, it was advisable at the outset of the work to run some comparative tests of the two gases.

Figure 4 shows the results of 13 series of experiments\* in which four fungi were exposed for different periods of time to various concentrations of nitrogen trichloride and chlorine. The curves represent minimum lethal dosages, all time-concentrations on and above the curves being lethal and all below ineffective or only partially effective. It will be noted

\* The writer was assisted in this part of the work by E. C. Raby and subsequently by D. S. Giddings.

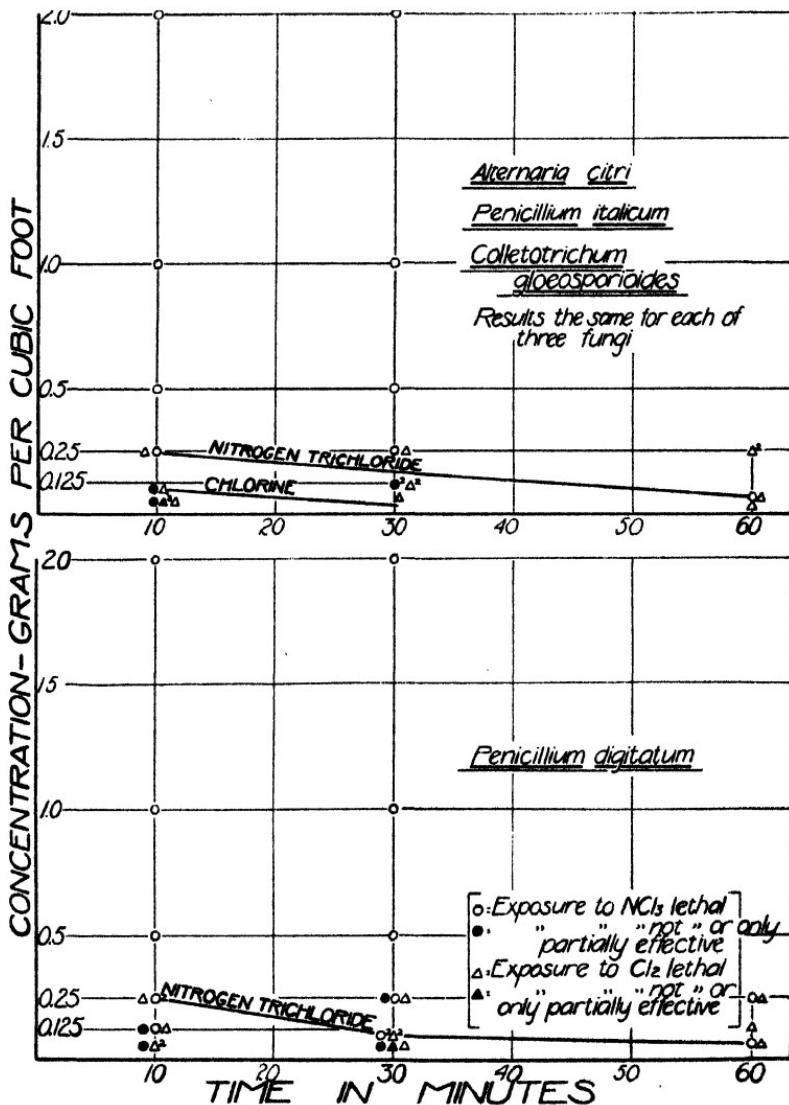


Fig. 4.—Lethal effect of several concentrations and exposures of nitrogen trichloride and chlorine upon four citrus fungi. The concentrations given here for the gases were those delivered to the treating chamber and do not represent the actual residual concentrations remaining in the air after the walls, shelves, etc., had been saturated with gas. Accurate means for the estimation of the concentration of the toxic gases in the air had not been perfected at this stage of the work.

that chlorine gas is more effective than nitrogen trichloride in killing pure cultures of the fungi.

However, trials on wounded and inoculated oranges, conducted thus far at the field laboratory of the California Fruit Growers' Exchange,

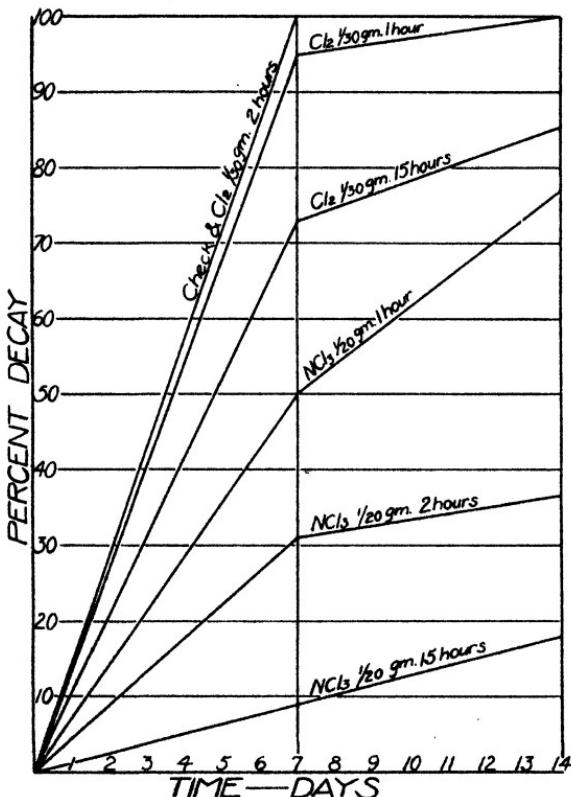


Fig. 5.—Comparison of nitrogen trichloride and chlorine in lessening decay in injured and inoculated Valencia oranges. The gases were delivered to the treating chamber in such concentrations that at the end of 15 minutes the concentrations shown were attained. The generator was then stopped and no additional gas introduced during the periods shown.

indicate that chlorine at the concentrations used was decidedly less effective than nitrogen trichloride in protecting the fruit from decay, the probable reason being that the chlorine injured the rind and made it more susceptible to the organisms of decay. This is shown in figure 5.

The concentration of  $\frac{1}{30}$  gram of chlorine per cubic foot was used because it caused no rind injury apparent to the eye. The reactivity of gaseous chlorine is several times as rapid as that of nitrogen trichloride; that is, chlorine coming in contact with moist surfaces, such as those of

the protoplasm of citrus fruits and molds, would react with such violent rapidity that it would be much more apt to injure and kill the cells than would nitrogen trichloride, which releases its reactive chlorine less rapidly. Moreover, because of its great chemical reactivity, chlorine may

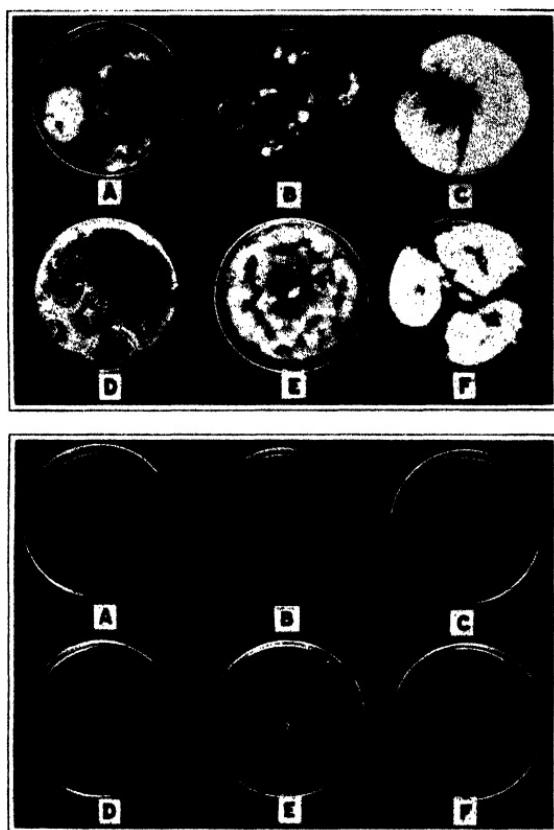


Fig. 6.—Above: Untreated plantings of *A*, *Colletotrichum gloeosporioides*; *B*, *Alternaria citri*; *C*, *Penicillium digitatum*; *D*, *Penicillium italicum*; *E*, *Phytophthora citrophthora*; *F*, *Botryosphaeria ribis*; after 4 days' incubation at 26° C. Below: Similar plantings treated exactly as those above but exposed to 30 mg per cubic foot of  $\text{NCl}_3$  gas for 2 hours.

decrease in concentration below the point lethal to fungus spores before all the infected surfaces are contacted.

*Nitrogen Trichloride*.—The toxicity of  $\text{NCl}_3$  gas to pure cultures of citrus fungi was apparent in the first series of experiments conducted. Figure 6 shows petri-dish cultures of *Colletotrichum gloeosporioides*, *Al-*

*ternaria citri*, *Penicillium digitatum*, *P. italicum*, *Phytophthora citrophthora*, and *Botryosphaeria ribis* that were left untreated as controls, and the corresponding six cultures that were killed by the gas.

Spore and mycelial suspensions of the organisms were made by pouring sterile water on agar slant cultures and loosening the growth with a

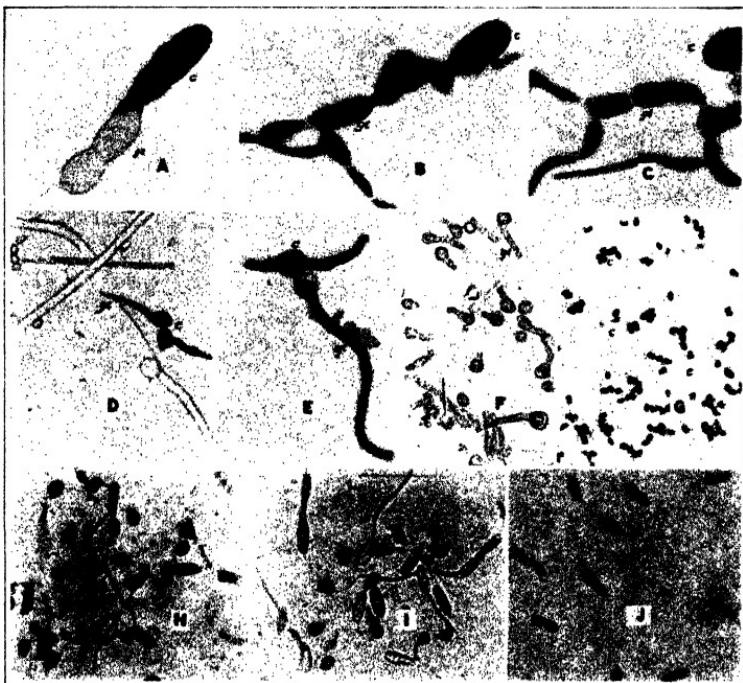


Fig. 7.—Effect of  $\text{NCl}_3$  on conidia (c) and germ tubes (gt) of several citrus fungi; A, control conidium and germ tube of *Alternaria citri*; B and C,  $\text{NCl}_3$ -gas-treated conidium and germ tubes of *Alternaria citri* showing shrunken protoplasts (A, B, C  $\times 600$ ); D, untreated germinating conidia of *Penicillium italicum*; E, F, and G, gas-treated *Penicillium italicum* with shrunken protoplasts; H and I, untreated conidia, germ tubes, and appressoria of *Colletotrichum gloeosporioides*; J, gassed, ungerminated conidia of *Colletotrichum gloeosporioides* (D-J,  $\times 260$ ).

platinum needle. Three drops of the suspension were placed aseptically on a sterile filter paper in a petri dish. After the closed dishes had been exposed to the gas for 5 minutes, the lids were set so as to cover a half or less of the bottom parts of the dishes, a rapid diffusion of the gas to the fungi thus being permitted. After treatment the cultures were returned to the laboratory and tested for viability by pouring on them melted glucose-potato agar at  $45^\circ \text{C}$  ( $113^\circ \text{F}$ ). The amount of, or the absence of, subsequent growth was the criterion of the effectiveness of treatment.

TABLE 2  
LETHAL EFFECT\* OF THREE CONCENTRATIONS OF  $\text{NCI}_3$  ON CONIDIA AND MYCELIUM OF CITRUS FUNGI†

Concentration of $\text{NCI}_3$ , mg per cu. ft.	Exposure hours	<i>Phytophthora</i> <i>citrophthora</i>		<i>Colletotrichum</i> <i>gloeosporoides</i>		<i>Alternaria</i> <i>citri</i>		<i>Penicillium</i> <i>digitatum</i>		<i>Penicillium</i> <i>italicum</i>	
		Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
0 (checks).....	....	-,-	+,+	+,+	+,-	+,-	+,-	+	++	++	++
6.25.....	{ 3	-	-	+,-	-	-	-	±,±	-	-	-
	{ 6	-	-	-	-	-	-	-	-	-	-
31.00.....	{ 3	-	-	-	-	-	-	-	-	-	-
	{ 6	-	-	-	-	-	-	-	-	-	-
105.00.....	{ 3	-	....	-	....	-	....	-	....	-	....
	{ 6	-	....	-	....	-	....	-	....	-	....

\* Explanation of symbols: + = growth; ± = growth, but much less than checks; - = no growth. Number of signs = number of petri-dish cultures tested.

† Results 48 hours after agar poured on treated fungi.

Microscopically the protoplasts of the spores, germ tubes, and hyphae treated with nitrogen trichloride were seen to be shrunken and pulled away from the cell walls; in some cases marked granular and coagulation effects were evident. These effects are shown in figure 7.

To determine the effect of three relatively low concentrations of  $\text{NCl}_3$  gas on five fungi that commonly attack citrus fruit, conidia and hyphae were placed on filter papers in petri dishes and exposed for 3 and 6-hour periods as shown in table 2. "Dry" means that the fungus was transferred directly without wetting from the test-tube cultures to the filter papers; "wet" means that a suspension of conidia and hyphae in water was the inoculum used.

Since the gas is only very slightly soluble in water, it was thought that possibly the water of the suspensions might protect the fungi. The results, however, show no such protection. In fact, the only evidences of growth were in 3 dry cultures. As a possible explanation of this incomplete killing, may be considered the fact that when the fungus is transferred dry, it is impossible to avoid hyphal and conidial clumps. These prevent an even distribution of the fungus and protect some cells from exposure to the gas. In the wet cultures the fungus is more easily broken up and distributed throughout the suspension. When the suspension is transferred, the water is quickly taken up by the filter paper, leaving the fungus unprotected and readily contacted by the gas. All the conidia planted on agar slants except those in the condensation water at the bottom of the slant are readily killed by the gas. Those protected by the water germinate and quickly cover the surface of the slant. It should be pointed out here that the surfaces of fruits when treated should be free from liquid water. The condensation water that forms on the rind of oranges coming from the precooler lessens the effectiveness of the gas treatment by preventing contact with some fungus spores.

Table 2 indicates that for a 3-hour period the minimum lethal dosage is less than 6.25 mg of the gas per cubic foot.

To determine the shortest exposure to a low concentration of  $\text{NCl}_3$  gas that will kill *Penicillium digitatum* and *P. italicum*, plantings of water suspensions of these fungi on filter paper were exposed to a concentration of 4.75 mg of  $\text{NCl}_3$  per cubic foot for the periods shown in table 3.

The data in table 3 indicate that an exposure of 20 or more minutes to the low concentration of 4.75 mg per cubic foot of  $\text{NCl}_3$  gas was lethal to the hyphae and conidia of *Penicillium italicum*. Exposures of 60 to 90 minutes were required to kill all the viable cells of *P. digitatum*, although some plates showed no growth of *P. digitatum* after a 20-minute exposure. The conidia of *P. digitatum* are less easily loosened from the

conidiophores than are those of *P. italicum*, which necessitates a more vigorous manipulation with the platinum needles and loosens more clumps of mycelium. Cells in the interior of these clumps are less apt to be contacted and killed by the gas.

Work similar to that discussed under table 3 was carried on with *Colletotrichum gloeosporioides* and *Alternaria citri* and was repeated with the two *Penicillium* species.

TABLE 3

EFFECT ON GROWTH\* OF *PENICILLIUM* spp. OF VARIOUS EXPOSURES TO 4.75 MG PER CUBIC FOOT OF  $\text{NCl}_3$  GAS

Duration of exposure, minutes February 5, 1934	<i>Penicillium digitatum</i>		<i>Penicillium italicum</i>	
	February 6, 1934	February 8, 1934	February 6, 1934	February 8, 1934
10.....	± 3 col.	± 3 col.	± 1 col.	± 16 col.
20.....	-	± 1 col.	-	-
30.....	-,-	± 1 col., -	-,-	-,-
40.....	± 3 col.	± 3 col.	-	-,-
50.....	± 1 col.	± 1 col.	-	-
60.....	± 1 col.	± 1 col.	-	-
90.....	-	-	-	-
120.....	-	-	-	-
150.....	-	-	-	-
180.....	-	-	-	-
Checks†.....	+, +, +, +, +	+, +, +, +, +	+, +, +, +, +	+, +, +, +, +, +

\* Explanation of symbols: + = growth; ± = growth, but much less than checks; - = no growth. Number of signs = number of petri-dish cultures tested.

† Checks had 50 or more colonies each.

The data of table 4 show that the *Penicillium* species, blue and green molds, are more easily killed by gassing than are *Alternaria citri* and *Colletotrichum gloeosporioides*. In making a spore suspension of the latter two fungi, it is very difficult or impossible not to include large clumps of mycelium, cells in the interior of which would be protected from the gas. With the *Penicillium* species, spore suspensions free from such clumps are more easily prepared. The data under "Conidial germination" in table 4 indicate that the spores of *Alternaria citri* and *Colletotrichum gloeosporioides* are likewise easily killed, provided the gas can contact them.

The success of the gassing method on Washington Navel and Valencia oranges led to attempts to adapt the procedure for the protection of lemons and grapefruit in storage and in transit. The latter two fruits are much more subject to injury by the gas than are oranges, which necessitates the use of lower concentrations. This injury is generally in the form of very minute pits which are rather noticeable on the light-colored rind of lemons and grapefruit. While the same type of injury

TABLE 4  
EFFECT ON GROWTH\* AND GERMINATION OF CITRUS FUNGI OF VARIOUS EXPOSURES TO 7.7 MG PER CUBIC FOOT OF  $\text{NCl}_3$  GAS

Duration of exposure, minutes	Culture No.	<i>Cercospora citricola</i>		<i>Alternaria citri</i>		<i>Penicillium digitatum</i>		<i>Penicillium italicum</i>	
		Mycelial growth after 4 days		Conidial germination after 4 days		Mycelial growth after 4 days		Conidial germination after 4 days	
		2 days	4 days	2 days	4 days	2 days	4 days	2 days	4 days
10	{ 1	-	-	-	+ 3 col.	-	-	-	-
20	{ 1	-	-	-	+ 1 col.	-	-	-	-
30	{ 1	-	-	-	-	+ 1 col.	-	-	-
40	{ 1	-	-	-	-	+ 1 col.	-	-	-
50	{ 1	-	-	+ 1 col.	-	-	-	-	-
60	{ 1	-	+ 1 col.	+ 2 col.	-	-	-	-	-
90	{ 1	-	-	+ 1 col.	-	+ 1 col.	-	-	-
120	{ 1	-	-	-	-	+ 2 col.	-	-	-
150	{ 1	-	-	+ 1 col.	-	+ 1 col.	-	-	-
180	{ 1	-	-	+ 1 col.	-	+ 1 col.	-	-	-
Checks	2	+	-	+ 2 col.	-	+ 3 col.	-	+ 50 col.	+
	3	+	-	+ 3 col.	-	+ 3 col.	-	+ 50 col.	+
	4	+	-	+ 3 col.	-	+ 3 col.	-	+ 50 col.	+

\* Explanation of symbols: - = no growth or germination; + = growth or germination; col. = colony; ± = slight growth.

TABLE 5  
EFFECT\* OF A VERY LOW CONCENTRATION (1.25 MG PER CUBIC FOOT) OF  $\text{NO}_2$  ON GERMINATION AND GROWTH OF CITRUS FUNGI, AUGUST 19, 1934†

Duration of exposure, hours	<i>Alternaria citri</i>		<i>Oospora citri-auranti</i>		<i>Penicillium digitatum</i>		<i>Penicillium italicum</i>	
	<i>Coleotrichum gloeosporioides</i>	Germination	Growth on agar	Germination	Growth on agar	Germination	Growth on agar	Germination
3.....	-	-	+	-	-	-	-	-
6.....	-	-	±	+	-	-	-	-
12.....	-	-	-	+ 1 col.	-	-	-	-
24.....	-	-	± <sup>1</sup>	-	-	-	-	-
0 (check).....	+	+ 3 col. or more	+	+ 3 col. or more	+ 50 col. or more	+ 50 col. or more	+ 50 col. or more	+ 50 col. or more

\* Explanation of symbols: + (with mycelia) = growth, + (with spores) = germination more than 50 per cent; ± = germination 1 to 10 per cent; ±<sup>1</sup> = germination less than 1 per cent.

† Results were recorded 48 hours after treatment.

occurs to some extent on oranges, it is scarcely discernible because of the darker color of the rind of that fruit. In addition to their tendency to pitting, lemons and grapefruit are more subject than oranges to a staining and brown discoloration due to overdoses of the gas. The work with lemons and grapefruit is still in the experimental stages. Table 5 indicates the effectiveness of a very low concentration of nitrogen trichloride (1.25 mg NCl<sub>3</sub> per cubic foot) on several citrus fungi. If it is found that lemons and grapefruit will tolerate such low concentrations of the gas for long periods, gas treatments should prove of great benefit in decreasing losses from decay of these fruits in storage.

**Ozone.**—The possibility of utilizing ozone as a sterilizer and protector of citrus fruits was also considered. This, however, was unsatisfactory because it was found that the gas at the concentrations<sup>7</sup> attainable with a small generator in a small chamber (3.646 cubic feet) at best only partially inhibited the germination and growth of the fungi on agar. When the cultures were removed from the gas chamber, even after 3 weeks' exposure, they resumed their usual rapid rate of growth. While in the presence of the gas, the fungi grew slowly within the medium and closely appressed to the surface, generating an alcoholic or ester-like odor and suggesting anaerobiosis. The cultures gave the iodoform reaction.<sup>8</sup>

Washington Navel oranges injured and then inoculated by immersion in a suspension of spores of *Penicillium digitatum* and *P. italicum* were not protected from decay when placed in ozone. The fungi failed to produce the characteristically colored fructifications while in the gas, except where the decayed rind was closely appressed to the floor of the gas chamber. However, the short, white, aerial hyphae destined to be conidiophores were in evidence on the surface of the fruits while in the presence of ozone, and within 16 hours after the stopping of the generator and the aeration of the chamber, the colored spores were in evidence. After 4 days at 25° C (77° F) the average diameter of the decayed area on the surface of fruits exposed to ozone was 103 mm, while the area on fruits allowed to decay in air was 114 mm, indicating but slight inhibition of the decay by the gas. Accordingly it was concluded that ozone in concentrations decidedly unpleasant and probably toxic to humans does not protect citrus from decay by the common blue and green molds.

Baker<sup>9</sup> obtained similar results with apples. His work indicated that

<sup>7</sup> The actual concentration of O<sub>3</sub> was not determined. It was sufficiently high, however, to give a strong, unpleasant odor and to cause headache, indicating toxicity to humans.

<sup>8</sup> Indicates presence of such substances as alcohols, acetone, or aldehydes.

TABLE 6  
EFFECT\* OF OZONE AND NITROGEN TRICHLORIDE ON CITRUS FUNGI (GERMINATION)†

Gas	Concentration, mg per cu. ft.	Hours exposed	<i>Coleotrichum gloeosporioides</i>		<i>Alternaria citri</i>		<i>Penicillium digitatum</i>		<i>Penicillium italicum</i>	
			Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Checks.....	.....	..	+, +	+, +	+, +	+, +	+, +	+, +	+, +	+, +
Ozone.....	.....†	{ 3 6 9	+ + +	+ + +	+ + +	+ + +	± ± +	+	± ±	+
NCl <sub>3</sub> .....	{ 6.25 6.25 105.00	3 6 2	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -

\* Explanation of symbols: + = more than 50 per cent germination; ± = less than 5 per cent germination; — = no germination.

† Concentration unknown; small generator run continuously in chamber of 12 cubic-feet capacity.

‡ Results 48 hours after treatment.

ozone has no effect on the appearance, general condition, flavor, and keeping quality of apples in storage.

*Comparison of Ozone and Nitrogen Trichloride.*—The results of an experimenter designed to compare the relative effectiveness of ozone and nitrogen trichloride are shown in table 6. Both dry spores from an agar slant culture and the wet spores of a water suspension were exposed and the effect on germination noted.

The concentration of the ozone was unknown, there being no dependable quantitative method of estimation available at the time. However, a comparison of ozone with the relatively low concentrations of nitrogen trichloride given in tables 2, 3, 4, 5, and 6 shows the relative ineffectiveness of the former gas. Only on the dry spores of the *Penicillium* species did the ozone give any evidence of inhibition of germination.

*Methylchloramines.*—Experiments with two other gases, monomethylchloramine ( $\text{CH}_3\text{NCl}_2$ ) and dimethylchloramine ( $(\text{CH}_3)_2\text{NCl}$ ), which presumably are less corrosive than  $\text{NCl}_3$  and therefore more easily adaptable to the treatment of lemons and grapefruit, are being initiated. Preliminary tests with  $\text{CH}_3\text{NCl}_2$  indicate that it is at least as effective as and possibly superior to  $\text{NCl}_3$  in sterilizing the surface of fruit. The cost of preparing  $\text{CH}_3\text{NCl}_2$  is more than that of  $\text{NCl}_3$ , however. A concentration of 20 mg of  $\text{CH}_3\text{NCl}_2$  per cubic foot for periods of 1 and 2 hours was lethal to *Penicillium italicum* and *Alternaria citri*.

*Sulfur Dioxide.*—Several packing-houses are using sulfur dioxide gas for sterilization of used boxes and for general disinfection purposes to lessen the hazards of reinfection, principally by brown rot and blue and green molds. Experiments are now in progress to determine the minimum concentrations of the chemical lethal to several citrus fungi. Exposure to a volume concentration of 1 per cent  $\text{SO}_2$  in air for 10 minutes was lethal to the brown rot fungus, *Phytophthora citrophthora*, but not to the blue and green molds, *Penicillium italicum* and *P. digitatum*. However, volume concentrations of 2 per cent or greater for 10 minutes were lethal to the two *Penicillium* species. Thus far no combinations of concentration of  $\text{SO}_2$  and period of exposure have been found which, without injuring the rind, will protect oranges from decay.

## SUMMARY

1. A gaseous mixture of low concentrations of nitrogen trichloride with air has been used successfully in controlling decay of Washington Navel and Valencia oranges due to *Penicillium italicum* (blue mold) and *P. digitatum* (green mold).

2. Procedures for the safe and effective use of the gas treatment in

storage rooms and refrigerator cars have been developed and commercial installations of apparatus have been made in the plants of fifteen packing companies.

3. After 3 to 4 weeks' storage, losses in oranges due to decay, as shown by numerous tests, may be reduced 50 to 75 per cent or more by giving the fruit three to five 3-hour treatments with 5 to 15 mg of  $\text{NCl}_3$  per cubic foot of air, at 3 or 4-day intervals, beginning the first day of storage.

4. Concentrations as low as 4 to 6 mg  $\text{NCl}_3$  per cubic foot for a period of 30 minutes were lethal to conidia of *Penicillium italicum*, *P. digitatum*, *Oospora citri-aurantii*, *Colletotrichum gloeosporioides*, *Alternaria citri*, and *Botryosphaeria ribis*, and to the mycelium of *Phytophthora citrophthora*.

5. Microscopically, the protoplasts of the gas-treated spores, germ tubes, and hyphae were seen to be shrunken away from the cell walls, in some cases with marked granular and coagulation effects.

6. Preliminary trials indicate that the method may be adapted to the treatment of grapefruit and lemons in storage by the use of lower concentrations of the gas for long periods.

7. Chlorine used in the same concentrations as nitrogen trichloride was injurious to the fruit and much less effective than the latter in decreasing decay. It was, however, more toxic to the fungi in cultures, and may be used for general disinfection of packing-houses and equipment.

8. Ozone showed only very slight or no toxicity to the organisms of decay and no protection of the fruit.

9. Preliminary tests indicate that monomethylchloramine may be substituted for nitrogen trichloride, but at greater cost.

10. Sulfur dioxide may be used effectively in the sterilization of boxes and for general disinfection purposes in the packing-house.

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## THE CYCLAMEN MITE, TARSONEMUS PALLIDUS, AND ITS CONTROL ON FIELD STRAWBERRIES<sup>1</sup>

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### INTRODUCTION

DURING THE PAST SEVERAL YEARS, strawberry fields in California have shown an increasing decadence, until, at the present time, a commercial planting may be expected to produce a crop of fruit for two years, or in some cases only one year, and then become commercially unproductive. This is strikingly in contrast with the conditions which existed 15 or 20 years ago, when, according to reports, strawberry plantings produced crops for 4 to 6 years. This decadence may be caused by one or more relatively obscure factors, of which the most important is, no doubt, in certain varieties at least, the virus disease known as yellows or xanthosis. In addition, strawberries suffer from accumulation of salts in the surface soil, and from root diseases of obscure etiology. Recently Thomas (1932) has discovered that the causal agent of a hitherto puzzling wilt disease is a fungus belonging to the genus *Verticillium*. Among the animal parasites of strawberries are the cyclamen mite, red spider, the strawberry root-worm, white grubs (larvae of various scarabaeid beetles), the stem nematode, the root-knot nematode, and aphids. The most important economically of these parasites of strawberries at the present time is the cyclamen mite, *Tarsonemus pallidus* Banks.

### IDENTITY OF THE SPECIES

The species which has been injuring strawberry plantings on the Pacific Coast and known to the growers as the strawberry mite has been identified for the authors by Dr. E. H. Ewing as the cyclamen mite *Tarsonemus*

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*pallidus* Banks. This species was named by Banks (1898), whose specimens were collected in a greenhouse in New York on leaves of chrysanthemums. About seven years later, Zimmerman (1905) described the strawberry mite, *T. fragariae* from specimens taken from strawberry plants growing out of doors in Austria. Since that time workers in Europe have known the species under the name *fragariae*, while in America the species has generally been called *pallidus*.

In the past, *Tarsonemus pallidus* and *T. fragariae* were believed to be distinct for several reasons, chief among which were the facts that cyclamen plants are not attacked in certain parts of the range of the strawberry mite and that until recently the strawberry was not attacked in the range of the cyclamen mite. Thus, in England, although the strawberry mite has been present for several years, no tarsonemids have been found attacking cyclamens, begonias, or other hosts of the cyclamen mite.<sup>4</sup> On the other hand, although the cyclamen mite has been in the United States since 1883 (Munger, 1933), no tarsonemid pest was discovered on strawberries until 1928 (Darrow, 1928).

Early attempts by the present authors to transfer mites from the strawberry to the cyclamen, and vice versa, were without success. Massee (1933) also described trials of this nature which were unsuccessful. Recently Ewing and Smith (1934), however, have successfully transferred mites from the strawberry to the cyclamen, and the present authors also have been able to complete the transfer to 92 per cent of the plants in one test, as described later.

This species is not readily transferred from one host to another. Floyd Smith<sup>5</sup> states: "I have frequently failed in getting *pallidus* established on strawberry and cyclamen, even when transferred from the same hosts. Although both these plants are favored hosts of *pallidus* in the East and are severely injured, they are not accepted under certain conditions." Massee (1933) states: "Experiments have been carried out at East Mall-ing to see whether the (strawberry) mites could be induced to live upon such plants as the Cyclamen, Begonia. . . . The investigations were extended over three seasons, from 1930-1933, but negative results were obtained every time." In 1930 the present authors transferred mites from the cyclamen to small cages fixed to strawberry leaves, and similarly from strawberry to cyclamen, but the mites died in every case. Again in the summer of 1934, 25 cyclamen plants were each infested with about 100 mites from strawberry, but in no case did they survive on the cyclamen. However, in the fall of 1934, another set of 25 cyclamen plants were each infested with about 50 mites from strawberry, and sub-

<sup>4</sup> Personal correspondence with A. M. Massee, September, 1934.

<sup>5</sup> Personal communication.

sequent examination showed strong colonies established on 92 per cent of the plants. Twenty-five check plants remained free from mites.

Ewing and Smith (1934) recently have made morphologic and biologic comparisons and conclude that no differences exist between *Tarsonemus pallidus* and the similar form on European strawberries. They list *T. fragariae* Zimm. as a synonym of *T. pallidus* Banks.

## HISTORY OF THE OCCURRENCE OF TARSONEMUS PALLIDUS ON STRAWBERRIES

The cyclamen mite has been observed on garden strawberries in Finland since 1892, by Reuter (Morstatt, 1908). Zimmerman (1905) first observed the mite in 1900 in Maerisch-Eisgrub, Austria, on garden strawberries, and it was subsequently recorded in Europe injuring field strawberries as follows: in 1906, Germany (Morstatt, 1908); 1906, Finland (Reuter, cited by Massee, 1930a); 1912, Norway (Schøyen, 1914); 1915, Sweden (Tullgren, cited by Massee, 1930a); 1916, Denmark (Ferdinandsen, 1919); 1924, England (Massee, 1930a); 1928, Russia (Savzdarg, 1928); 1928, Switzerland (Osterwalder, 1928); 1930, Holland (Sprenger, 1930).

In the United States the first observation of this species attacking field strawberries was that of Darrow (1928). A simultaneous discovery was made by M. B. Davis in Ontario, Canada (Darrow, 1928). In 1928, Darrow discovered the mite in strawberry fields in Maryland, New York, and Massachusetts. In response to a questionnaire the present authors were informed that this species also had been found injuring strawberries in Wisconsin, Washington, and Oregon. From data furnished by J. A. Hyslop, of the United States Department of Agriculture Insect Pest Survey, supplemented by replies to the questionnaire, the cyclamen mite is now known to occur (on various hosts both out of doors and in greenhouses) in the following states: Washington, Oregon, California, Montana, Colorado, Nebraska, Kansas, Texas, Minnesota, Iowa, Missouri, Wisconsin, Illinois, Mississippi, Kentucky, Michigan, Ohio, Alabama, Virginia, Pennsylvania, Maryland, New Jersey, New York, Massachusetts, Connecticut, Delaware, and New Hampshire.

In California the cyclamen mite occurs in most of the major berry-producing areas, where varieties acceptable to mites are grown. The greatest economic injury occurs in the coastal belt in the vicinity of Salinas, Watsonville, and Santa Cruz, and in the Santa Clara Valley. Some injury is also produced in the areas around Los Angeles and San Diego. Although susceptible varieties are grown inland in the southern part of the state, near Riverside and in the Imperial Valley, no mites could be found in these sections; and it seems likely that the high tem-

peratures or low humidities of summer annually exterminate the mites which may be brought into these areas.

### LIFE HISTORY

In their development to mature males and females, the mites pass through the egg, larval, and "pupal" stages. The eggs (fig. 1, *B*) are white, opaque, smooth, ovoidal, and unusually large when compared with the size of the adult mites. They are approximately 125 microns in length and 75 microns in diameter. The egg stage was found to last three or four days at about 20° C. Savzdarg (1928) states that the egg stage requires 9 to 13 days at a temperature of 15°–18° C. The shell is very thin and the embryo is clearly visible in the egg for some time before hatching.

The eggs hatch into six-legged larvae, with the first two pairs of legs situated close to the head and the third pair on the posterior half of the body. The larvae are opaque white, and have at the posterior end of the body a peculiar triangular enlargement. Larvae average approximately 200 microns in length and 80 microns in width. At room temperatures the larval period was completed in from 1 to 4 days, with an average duration of 2.8 days.

When the larvae are fully grown the larval integument becomes loosened from the body, but still covers it to form a "pupal" case. The legs are withdrawn from their integuments and lie in the loose skin of the larval body. The two anterior pairs of legs are directed anteriorly and closely appressed to the head while the third pair of legs is directed to the rear. A fourth pair of legs appears in this stage, posterior to the third pair of the larva. This form, or "pupa," is without means of locomotion. "Pupae" are approximately 225 microns long and 95 microns wide. The "pupal" stage lasts for 2 to 7 days, with an average duration of 3.8 days.

At the completion of the "pupal" stage, the larval skin splits and the adult mite emerges. The adults are of two distinct structural types: the female and the male. The body of the adult female (fig. 1, *A* and *C*) is regularly oval, with a relatively large, distinct, nearly heart-shaped capitulum projecting anteriorly. There are four pairs of legs. The first and second pairs are situated close to the head while the third and fourth pairs are on the posterior half of the body. The fourth pair of legs of the female are reduced to thin, rod-like structures which project to the rear and are not used for walking. At the apex, the fourth leg carries one long and one short bristle. Each of the other legs terminates in a retractile bladder-like structure which is expanded when the leg is in contact with the substratum, and is retracted while the leg is carried

forward. The two sides of the bladder are heavily chitinized to form a sheath over the retracted structure. When the bladder is expanded these chitinizations resemble claws. Each leg of the anterior pair possesses, in

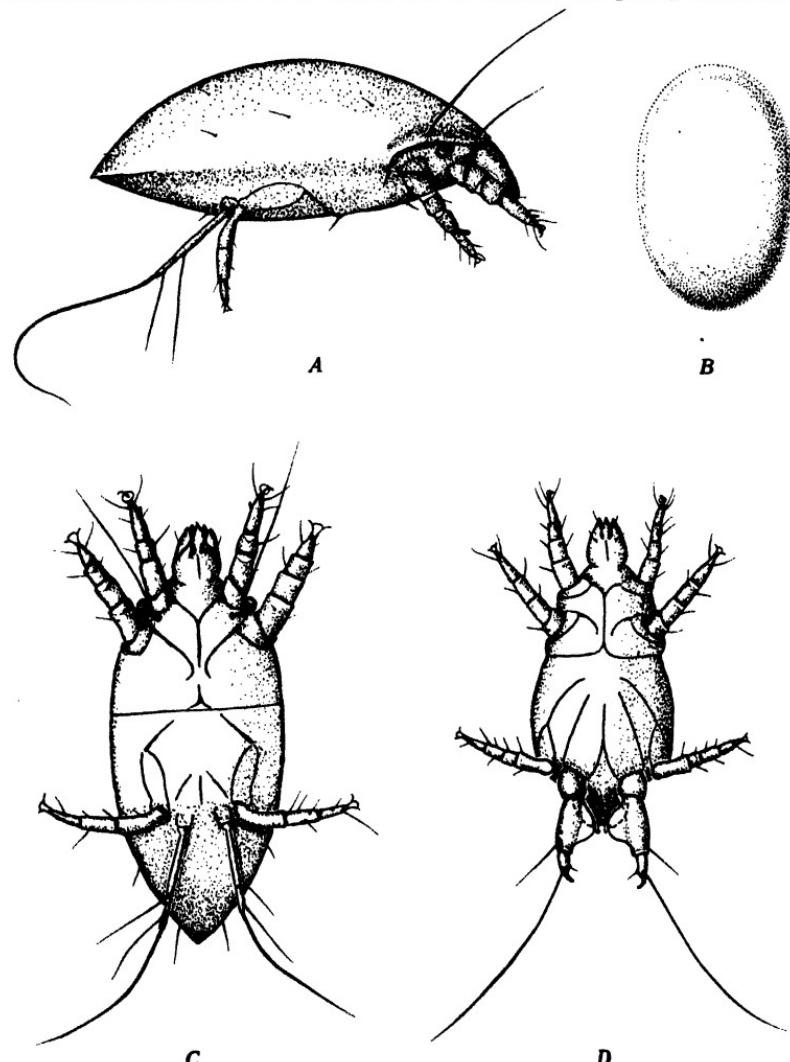


Fig. 1.—*Tarsonemus pallidus* Banks: A, adult female, lateral view; B, egg; C, adult female, ventral view; D, adult male, ventral view. All at same magnification.

addition, a median, single claw. On either side of the body between the first and second pairs of legs occurs (in the adult female only) the so-called clavate organ. This is apparently a solid sphere which is supported

by a stalk attached to the body in a cup-like formation of the integument. When cross sections of the mites were examined it was found that the whole clavate organ is situated in a deep pocket between the dorsal shield and the bases of the first and second legs, so that the apex of the sphere is scarcely visible beyond the edge of the dorsum. Adults which have newly emerged from "pupae" are pale-amber-colored but as they become older they darken. Old adults are sometimes brown. The integument glistens as though covered with oil. While the legs are provided with numerous bristles and a few clavate hairs, the body is nearly devoid

TABLE I  
LENGTH OF STADIA OF THE CYCLAMEN MITE ON VARIOUS HOSTS, IN DAYS

Stadium	Geranium 20°-25° C*	Cyclamen 18°-21° C†	Strawberry 16°-21° C	Average
Egg.....	5.0	11.0	3.5	6.5
Larva.....	2.3	7.0	2.8	4.0
"Pupa".....	2.0	3.5	3.8	3.1

\* Data averaged from Garman (1917).

† Data from Mozzette (1917).

of vestiture. Females average about 230 microns in length and 120 microns in width. The thickness of a few specimens which could be measured averaged about 80 microns.

The adult male (fig. 1, D) resembles the female in color and vestiture, but is smaller. In the male, the legs of the posterior pair are considerably enlarged, and are not used in locomotion. The penultimate segment bears a membranous dilation which projects mesad. Males average 170 microns in length and 95 microns in width.

The technique used for rearing was that described by Garman (1917), with pieces of strawberry leaves substituted for geranium. Garman reared the mites on geranium at a temperature of 20°-25° C; Mozzette (1917) reared them on cyclamen at a temperature possibly ranging from 18° C to 21° C; and the present authors reared them on strawberry at a temperature ranging from 16° to 21° C. The results of these three rearings are summarized in table 1.

#### HABITS AND ENVIRONMENTAL REQUIREMENTS

The cyclamen mites avoid light. They are rarely found on exposed surfaces of the plant. The majority of mites always occur in the unopened leaflets in the center of the crown. Here they are usually found between the two closely appressed halves of the dorsal surface of the folded leaflet. In this situation the colony of mites is usually located at the base of the midrib near its junction with the petiole of the leaf (fig. 2). Mites

are also found on the outer surfaces of the folded leaflets when these leaflets are closely appressed. This is especially the case in varieties which have dense pubescence on the ventral leaf surface, as in *Fragaria chiloensis*. Mites, in all stages of development, are also commonly found in strawberry flowers. Here they generally occur at the bases of the

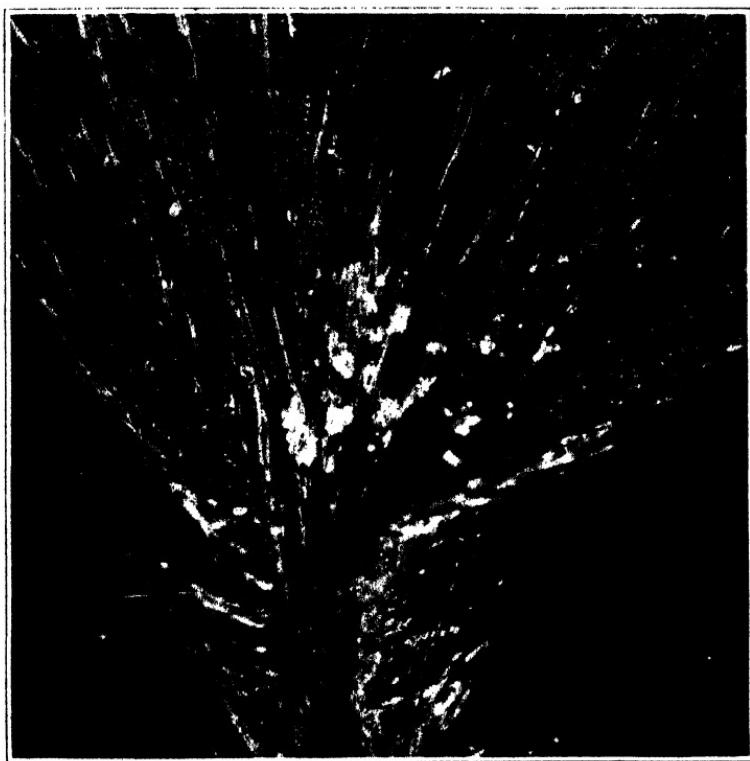


Fig. 2.—Upper surface of a young leaflet of Nich Ohmer strawberry which has been opened to show mites and eggs at the base of the leaflet.

petals and on the inner bases of the sepals. In cases of heavy infestation, all stages of mites can be found distributed over the green seeds of the immature berry, but protected by the dense formation of the styles. After the styles dry, mites can no longer be found on the fruit.

The cyclamen mite requires a high humidity. In rearing the mites in micro-cages in the laboratory it was found that the mites died when the humidity in the cage was allowed to decrease; and that the mites survived and developed better when the humidity was near saturation. Garman (1917) states that *Tarsonemus pallidus* "prefers a high relative

humidity (80%–90%) and a temperature ranging from 60°–80° F." Plants wet with rain and dew have been examined and mites have been found alive in droplets of water, indicating that they may survive submergence, for hours. In control experiments (discussed later) wherein mites were immersed in hot water, a check was generally conducted by submerging mites in cold water for two hours. This submergence was without injurious effect on the mites.

The cyclamen mite can exist through a wide range of temperature. Adult females and larvae lived for 3 days on strawberry leaves which were allowed to freeze in a beaker of water. At San Jose the authors have frequently seen the mites endure subfreezing temperatures during the winter. On a few occasions the temperature dropped to 17° F without killing the mites. On the other hand (as indicated later in control tests), prolonged exposure to a temperature of 100° F proves lethal to the mites. Furthermore, the authors were unable to find the mite in strawberry plantings in the inland section of southern California where summer temperatures frequently exceed 100° F; but whether these high temperatures prove lethal, or whether the coincident low humidity is the lethal factor could not be determined.

Adult females overwinter in a true condition of hibernation in the crowns, at the bases of the petioles of the leaves. In the strawberry plant the petiole of the leaf becomes flattened, crescentic in cross section in the basal half inch of its length, and is applied closely against the stem of the plant and bases of other petioles. On either side of the flattened portion is borne a large foliaceous stipule which often attains a length of 1½ inch. The stipules soon die and become brown and paper-like. They likewise ensheathe the crown. The inner base of the petiole is glabrous; the outer portion and the stipules are pubescent. Adult females are found during the winter between the glabrous inner surface of one petiole and the pubescent outer surface of the one above it. This situation is usually above the level of the soil, but is occasionally covered. On heavily infested plants 10 to 15 hibernating females may be found on one petiole. A limited amount of brown scarring indicates that normally little feeding is done during hibernation.

Females emerge from hibernation in the spring. The time of emergence apparently varies considerably with the mildness or severity of the weather. Observations during the past five years indicate that females usually leave their hibernating quarters about the latter part of February. They migrate to the center of the crown and commence oviposition at this time. Only adult females can ordinarily be found during the winter; but during the winter of 1933–34, a phenomenally mild winter, oviposition was carried on through the late fall and winter

until the middle of January, at Watsonville. There occurred a short period in the latter part of January and early February when eggs could not be found, although "pupae" and males each constituted about 5 per cent of the total population during this period. Hence, it seems likely that during exceptionally warm winters the mites may breed throughout the year in central California.

The rate of oviposition is unusually rapid when the relation of the size of the egg to the size of the female is considered. Isolated females in cages, fed on small pieces of strawberry leaves, deposited 3 eggs a day for 4 consecutive days. Although the average rate of egg deposition falls below 3 a day in cage tests, it seems likely that this rate may be maintained when mites are in their normal environment. Parthenogenesis of these eggs was proved by Garman (1917). He reared several generations without the appearance of males. In the field, males do not normally overwinter. They appear in very limited numbers in May and increase slowly until the advent of cold weather in the fall. At this time, in colonies containing many "pupae," they may number 30 per cent of the total population. On the average, males probably constitute about 5 per cent of the total population.

The modified fourth pair of legs of the male is used as a pair of claspers with which to pick up "pupae"; males are frequently seen carrying them about, with the anterior end of the "pupa" to the rear. Males are occasionally seen holding mature females in the same manner. They can be distinguished from females with a 14-power hand lens, chiefly because of their greater activity and faster rate of walking. The smaller size and enlarged fourth pair of legs also aid in recognition of the male.

#### HOST PLANTS

The cyclamen mite attacks many species of plants, other than strawberries (*Fragaria*). Floyd Smith (1933) lists the following plants attacked, for the most part, in greenhouses: *Amaranthus retroflexus*, pigweed; *Antirrhinum majus*, snapdragon; *Begonia semperflorens*, wax begonia; *Capsicum annuum*, common red pepper; *Chrysanthemum frutescens*, marguerite; *Chrysanthemum hortorum*, common chrysanthemum; *Crassula rubicunda*; *Cyclamen indicum*, cyclamen; *Dahlia rosea*, old garden dahlia; *Delphinium ajacis*, rocket larkspur; *Delphinium* sp., a hybrid of belladonna larkspur; *Fragaria* sp., strawberry; *Fuchsia speciosa*, common fuchsia; *Galinsoga parviflora*; *Gerbera jamesonii*, flame-ray gerbera; *Impatiens sultani*, sultan snapweed; *Lantana camara*, common lantana; *Oxalis* sp., wood sorrel; *Parthenocissus tricuspidata*, Boston ivy; *Pelargonium peltatum*, ivyleaf geranium; *Pelargonium hortorum*, fish geranium var.; *Petunia hybrida*, common petunia; *Rubus*

sp., blackberry; *Verbena* spp., common verbenas; *Veronica peregrina*, purslane speedwell.

In addition to some of the plants mentioned above, Munger (1933) lists as hosts: forget-me-not, African violet, sweet pea, larkspur, cybodium, moccasin flower, rhododendron, orchid, heliotrope, stevia, daisy, and gloxinia.

It should be borne in mind that the cyclamen mite is primarily a greenhouse pest and that practically all of the above-mentioned hosts

TABLE 2  
PLANTS TESTED AS HOSTS OF *TARSONEMUS PALLIDUS*

Species	Number of plants used	Number of plants infested 72 days later	Species	Number of plants used	Number of plants infested 72 days later
<i>Fragaria cuneifolia</i> (Mariposa, Calif.).....	17	7	<i>Fragaria californica</i> (Mariposa, Calif.).....	16	9
<i>Fragaria chiloensis</i> (Pigeon Point, Calif.).....	8	2	<i>Rubus parviflorus</i> .....	5	0
<i>Fragaria chiloensis</i> (Chile).....	3	2	<i>Rubus vitifolius</i> .....	8	0
<i>Fragaria</i> sp. (China).....	2	2	<i>Potentilla lindleyi</i> .....	2	0
<i>Fragaria californica</i> (Alma, Calif.).....	3	1	<i>Potentilla glandulosa</i> .....	10	7
			<i>Geum</i> , sp. ....	4	3
			<i>Acaena microphylla</i> .....	7	0

have been infested under greenhouse conditions. In response to a questionnaire addressed to entomologists in each of the several states, only two records of the occurrence of the cyclamen mite out of doors were obtained (exclusive of the occurrence of this species on strawberry). Both of these records pertained to occurrence on *Delphinium*.

The authors attempted to determine whether or not under out-door, California conditions, other plants than strawberries were hosts of the mite. Two types of plants were tested: wild species of *Fragaria*, and rosaceous plants closely related to the genus *Fragaria*, which may occur at times near berry-producing areas. The plants were grown in pots in an open lath house at San Jose. Each plant was infested in May by placing small leaflets from infested strawberry plants deep into the crowns of the test plants, until approximately 75 to 100 mites had been colonized on each plant. Seventy-five days later the plants were examined and the data, given in table 2, recorded. The majority of the infested native *Fragaria*, *Potentilla*, and *Geum* plants were severely injured and some were completely killed by the mites. No mites survived the winter on any of these potted plants, and have not been observed on any of these species growing under natural conditions.

All commercial varieties of strawberries are hosts of the cyclamen

mite. However, in some sections certain varieties are much more susceptible to mite attack than certain other varieties which escape commercial injury. Various authors have indicated differences in susceptibility as shown in table 3.

In variety trial plots at San Jose, observations in the fall of 1930 and of 1931 indicated that the varieties grown in the open were infested as shown below:

<i>Severely Attacked</i>		
Aberdeen	Ford	Mascot
Big Joe	Fruitland	Mastodon
Big Late	Giant 999	Missionary
Blakemore	Haverland	Nich Ohmer
Boquet	Kellogg's Premier	Paul Jones
Buback	King Wealthy	Princee
Champion K	Klondike	Red Gold
Chesapeake	Lord Salisbury	Sample
Eaton	Lucky Strike	World Wonder
<i>Slightly Attacked</i>		
Beacon	Gandy	Stevens Late Champion
Bliss	Kaliene	St. Louis
Clarke Seedling	Marvel	Superb
Cooper	Progressive	Washington
<i>Not Attacked</i>		
Aroma	Burgess	Senator Dunlap
Banner	Early Bird	Wild ( <i>Fragaria californica</i> )
Booster	Marshall	Wild ( <i>Fragaria chiloensis</i> )
Brandywine	New Oregon	

In considering the varieties which were found to be free of mites as given in the above list, it should be pointed out that both *Fragaria californica* and *F. chiloensis* were found to be acceptable to mites when infested by hand (table 2). Furthermore, commercial plantings of Brandywine and Aroma plants were found to be infested at Watsonville. The varieties Early Bird, Burgess, Booster, and Senator Dunlap are not grown commercially in California and hence have not been definitely proved to be resistant. The varieties Marshall, New Oregon, Oregon Plum, and Banner are either identical or very similar. Henceforth in this paper, all will be indicated by the name Marshall. This variety is resistant in central California.

The chief commercial varieties grown in California are the Marshall and Nich Ohmer in the northern and central part, and the Klondike in the southern part of the state. In addition, the varieties Mastodon, Capitola, Magoona, Blakemore, and Brandywine have been grown to a limited extent in the north, while a few Missionary, Blakemore, Nich Ohmer, and Champion K have been grown in the south. All of these varieties

have been found to be injured by the cyclamen mite, in commercial fields. The major loss however, occurs with the variety Nich Ohmer in the coastal portion of central California, particularly in the vicinity of Watsonville, Salinas, and San Jose. During the past five years no serious injury has been done to Marshalls by the cyclamen mite, in this section (with one exception), although Marshalls adjacent to heavily infested Nich Ohmer patches are generally found to support a few mites.

TABLE 3

VARIETIES OF STRAWBERRIES REPORTED AS SUSCEPTIBLE TO ATTACK  
BY *TARSONEMUS PALLIDUS*

Varieties heavily attacked	Varieties slightly attacked	Location and authority
Deutsch Evern.....	Roskilde	
Sejherren.....	Victoria	
Dybdahl.....	Rubezahl	
Laxton's Noble.....	Ohne Bedenken	
Konigin.....	Purpurkugel	
Louise.....	.....	
Abundance.....	.....	
Bedford Champion.....	.....	
American Everbearing.....	Sieger	
Dresden.....	.....	Germany (Naumann, 1924)
Royal Sovereign.....	.....	England (Massee, 1930b)
Mastodon.....	Dr. Burril	
Vanguard.....	Premier	
Parsons.....	.....	Canada (Ross and Caesar, 1929)
Mastodon.....	Marshall	
Clark.....	.....	
Improved Clark.....	.....	Washington State (Arthur Hansen, personal communication)

In the southern part of the state the variety Klondike is grown almost exclusively. The usual practice in this area consists of harvesting a large crop in the first crop year and a second smaller crop in the second crop year, after which, because of decrease in size of fruit, the field is disked out. This type of culture, and the low humidity which prevails during the summer in most of the berry-producing sections of the south apparently combine to reduce the injury of the cyclamen mite to a point where it is of slight economic importance on Klondike.

#### INJURY AND SYMPTOMS ON THE STRAWBERRY PLANT

The symptoms produced on the strawberry plant are so pronounced that it is difficult to believe that the few mites usually present could have produced them. In order to definitely determine the effect of the mites, 24 Nich Ohmer plants were infested with either 5 or 20 females each,

in the middle of February. Twenty-four noninfested plants from the same source were held as a check. Six Marshall plants were likewise infested and 6 held as checks. The check plants remained free from mites

TABLE 4

## COURSE OF MITE INFESTATIONS AND INJURY ON STRAWBERRY PLANTS, 1931

Plant No.	Mites used for infestation February 18	Observations, May 23*			Observations, June 30		
		Eggs	Larvae and "pupae"	Adults	Degree of infestation	Degree of infestation	Injury
Nich Ohmer							
1	5	+	+	+	Light	Very heavy	Medium
2	5	+	+	+	Medium	Heavy	Medium
3	5	+	+	+	Medium	Light	Heavy
4	5	+	+	+	Heavy	Light	None
5	5	+	+	+	Heavy	Light	Heavy
6	5	+	+	+	Heavy	Heavy	Medium
7	5	-	-	-	None	Medium	None
8	5	+	+	-	Very light	Very heavy	Light
9	5	-	-	-	None	Medium	None
10	5	+	+	+	Heavy	Heavy	Heavy
11	5	+	+	+	Medium	Medium	Medium
12	5	+	+	+	Medium	Heavy	Medium
13	5	+	-	-	Very light	Heavy	Light
14	5	+	+	+	Light	Very light	None
15	5	+	+	+	Medium	Very heavy	Heavy
16	5	+	+	+	Heavy	Light	Medium
17	5	+	+	+	Heavy	Very heavy	Heavy
18	5	+	-	+	Light	Light	None
19	5	+	+	+	Heavy	Very heavy	Heavy
20	5	-	-	-	None	Medium	Light
21	20	+	+	+	Heavy	Light	Heavy
22	20	+	+	+	Very heavy	Medium	Heavy
23	20	+	+	+	Heavy	Heavy	Light
24	20	+	+	+	Heavy	Very light	Light
Marshall							
25	20	+	+	+	Light	None	None
26	5	+	+	+	Medium	Heavy	Medium
27	5	+	+	+	Light	Medium	Medium
28	5	+	+	+	Light	Medium	None
29	5	+	+	+	Medium	None	None
30	5	+	+	+	Medium	Medium	Light

\* Plus sign, stages present; minus sign, stages absent.

throughout the year and indicate that all plants were clean at the beginning of the test. The observations on the infested plants are recorded in table 4. From this table it is evident that 5 females are capable of establishing large colonies in a period of three months, since plants which were classified as heavily infested on May 23, supported from 200 to 500 mites in all stages. Three plants appeared to be free of mites

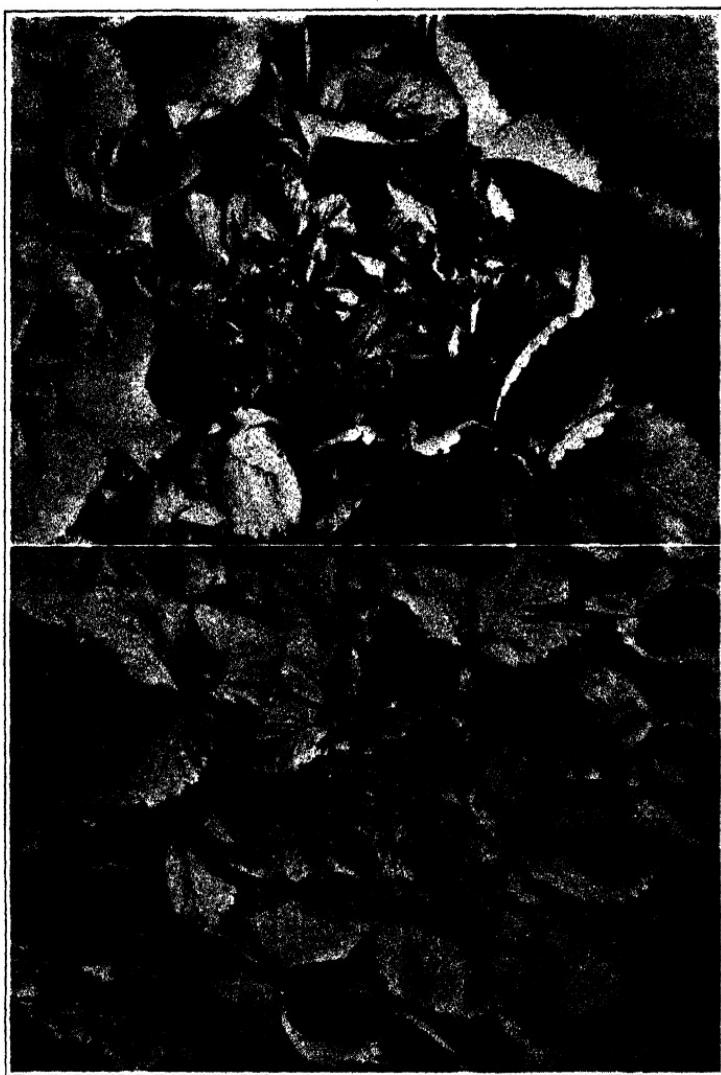


Fig. 3.—Strawberry plants showing advanced symptoms of injury. Upper, variety Nich Ohmer; lower, Boquet. (Photograph by Harold Thomas.)

on May 23 which indicates that a high percentage of mortality occurred among the 5 original mites placed on each plant. The differences in general infestation on May 23 are probably the result of the death of some of the original females.

Symptoms of injury were apparent on 22 of the 30 plants, four months after infesting. "Heavy" injury consisted of severe dwarfing and stunting; "medium" injury consisted of wrinkled leaves and may be considered as of economic importance. These tests indicate that a

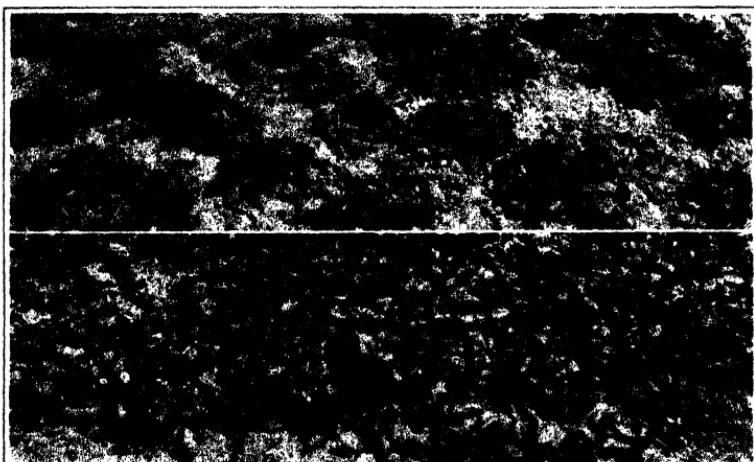


Fig. 4.—Upper: Nich Ohmer plants dwarfed by mites. Lower: plants of the same variety not infested. Both photographs taken in commercial fields, at the same time, in the fall of the first crop year.

very few females, possibly 1 or 2 per plant, are capable of giving rise to progeny sufficient to severely injure the plants in a single growing season.

Deviations from the normal course of infestation, as indicated by plants 4, 5, 16, and 29 are discussed under "Natural Control."

Symptoms of mild injury on the Nich Ohmer are indicated by a roughened, wrinkled appearance of the upper surfaces of the leaves, together with an irregular folding and fluting of the leaf margin. Upon closer inspection, the areas on the upper surface of the leaf between some of the larger veins are seen to be bulged upward and when these bulges are examined with a hand lens they somewhat resemble blisters. In cases of mild injury the plant assumes a dense appearance, as a result of the failure of the petioles to elongate properly. In such cases the wrinkled leaf blades remain nearly vertical in the crown, and crowded together, rather than assuming the normal horizontal position.

Symptoms of severe injury (figs. 3 and 4) consist of a pronounced dwarfing of the center of the crown. Small leaflets do not unfold completely and attain less than one-tenth of their normal size. Petioles are much reduced. Small, severely injured leaflets become pale, yellowish green in color, and of a hard, brittle texture. Finally, many small leaflets are killed in the crown before they unfold. These turn brown and appear somewhat silvered by the dense pubescence on the exposed underside leaf surfaces. Infested flowers and young fruits are brown near the inner bases of the sepals, and in severe cases entire flowers and young berries turn black and dry up.

Symptoms on other varieties are similar, with the exception that in some, particularly Klondike, the bases of the midribs turn reddish brown.

All of these symptoms are believed to be the result of feeding punctures made by the mites, which puncture the tender growing tissues with their styliform mouthparts and extract the plant protoplasm. This results in minute dead areas which fail to grow with the other expanding portions of the leaf, so that bending and twisting of the leaf occur.

Several investigators (Harmsen, 1934) have conducted similar trials to determine the extent of damage caused by the mite, and at times, it seems that the injury is greater than the number of mites present could produce. Thus Massee (1930b and 1933) hand-infested 12 Royal Sovereign plants and, after symptoms had developed, eradicated the mites; five of the plants died. He suggested that a virus may be associated with mite attack. The present authors believe, however, that in those cases wherein severe symptoms occur and mites are scarce on the plant, some factor such as predators or climate has intervened to control the mites (see discussion under "Natural Control").

In commercial strawberry plantings the mite usually becomes of economic importance in the fall of the first crop year (that is, when the planting is nearly two years old), and by the fall of the second crop year all of the plants are dwarfed and the field is entirely unproductive. When, however, planting stock is obtained from a heavily infested field, the injury may be severe by fall of the first growing year, and the field may have to be abandoned by the middle of the first crop year.

#### INTRODUCTION AND DISTRIBUTION OF MITES IN THE STRAWBERRY FIELDS

The primary method of introduction of the mites into new strawberry plantings consists of planting infested nursery stock. Mites have not been found on shipments of planting stock, but because of their minute size and the limited time spent in such inspection, failure to find them is

not surprising. On the other hand, clear-cut cases of mite introduction on planting stock have been seen in the commercial berry fields.

Before cases resulting from infested planting stock can be recognized, it is necessary to determine the extent of infestation which will result from natural spread from old infested fields, into adjacent, clean plantings. In the fall of 1933 several ranches were studied to determine the rate of natural spread. In the optimum case examined new Nich Ohmer plants had been set on about five acres, separated by only a narrow road from an old, heavily infested four-acre field of Nich Ohmers. The prevailing wind blew from the old field into the new. The latter field was mapped in the fall of the first year, by examining two rows, then skipping five and again examining two rows and skipping five, etc. Every mother or daughter plant which showed symptoms of mite injury was recorded. When the area was divided into four equal strips parallel to the old field it was found that in the strip adjacent to the old there were 35 mother plants and 7 daughter plants which showed symptoms of mite attack. In the next quarter there were 7 mother plants; in the third quarter 7; and in the fourth, and most remote quarter, there were 4. Assuming that 8,000 mother plants were planted to the acre, the total of 53 mother plants amounts to 0.13 per cent of mother plants infested by fall of the first growing season. The distribution of infested plants in the new field and the presence of infested daughter plants attached to noninfested mother plants, indicate clearly that the planting stock used in this field was free from mites when planted.

A number of berry fields were examined which showed unmistakably that mites had been brought in on the planting stock. One of the clearest cases consisted of about three acres planted to Nich Ohmers. Half of the field was planted with plants from one source while the other half was planted with plants from a different source. Mother plants in one-half of the field averaged 0.4 per cent infested, while in the other half they averaged about 30 per cent infested. All other factors such as situation, time of planting, etc., were uniform for the two lots. There is no doubt that the first lot of plants was free from mites when purchased, whereas the second lot was infested.

After the mites are once established they spread rapidly from plant to plant. This distribution is accomplished by (1) spread along runners, (2) transported on the bodies of insects, (3) blown by wind, and (4) carried by pickers, baskets, irrigation water, etc.

Mites reach daughter plants from an infested mother plant either by living in the terminal bud of the elongating runner, or, probably more frequently, by walking along the runners to the daughter plant, after the formation of the latter. Munger (1933) found that the mites could

crawl about half an inch a minute. At this rate a mite could crawl from the mother plant to a daughter plant, via a runner, in about half an hour. In strawberry fields where but a limited number of mother plants were originally infested, it is generally found that all of the daughter plants attached to an infested mother plant are infested while most of the adjacent plants are free from mites.

Mites are doubtless spread from flower to flower by bees and other insects which visit the strawberry flowers. In heavy infestations a single flower may contain several hundred mites in all stages of development. There is every reason to believe that bees may successfully transfer any stage of mites, including eggs, from infested to noninfested flowers.

Mites have been shown to be wind-borne, by Dustan and Matthewman (1932) who succeeded in catching them on tanglefoot screens in infested areas. Finally, mites are probably distributed in the field by the various human agencies, such as on the hands and clothing of pickers, on baskets and crates, on hoes, and probably to a minor extent by irrigation water.

By these various means a very rapid distribution occurs, once the mites are established. Cases have been noted wherein the infested plants in the fall of the first year approximated 0.5 per cent of all the plants. A year later, in the fall of the second year, these fields generally show 100 per cent of the plants infested and giving evidence of injury.

#### NATURAL CONTROL

A large amber-colored predatory mite, identified by H. E. Ewing as *Seiulus* sp., feeds on the cyclamen mite on strawberries in all parts of California. This species is often responsible for considerable destruction of the cyclamen mite, and in rare instances, coupled with unfavorable breeding conditions for the mite, this predator has reduced its numbers nearly to extinction. In such a field *Seiulus* reach their maximum numbers. They are often twice as numerous as the cyclamen mites. Because of their smaller size the cyclamen mites enter small interstices in the folded leaflets which the predators cannot enter. Conditions which favor an increase of predators at the expense of cyclamen mites are those which retard the production of new leaflets in the center of the crown, while the leaflets already formed open out to expose the contained cyclamen mites. The resistance of certain varieties, such as the Marshall, to mite attack may be due to the fact that their leaflets open more rapidly and thus facilitate biological control.

In Nich Ohmer fields of normal vigor the predators and cyclamen mites reach an oscillating balance, in which the cyclamen mites increase for a time, then decrease under the attack of the predators; this change in population trend may occur two or three times during a single season.

In spite of these periodic reductions in their numbers, however, the cyclamen mites are able to render the planting unprofitable in one or two years.

The effect of predators can be seen on the experimentally infested plants, reported in table 4. Plant number 4, for instance, showed a heavy infestation in May but a light infestation in June. The same is true of plants 5, 14, 16, and 21. Predators were found on some of these plants and probably occurred on all. It appears that among the Marshalls, predators became established on plants 25 and 29.

#### ARTIFICIAL CONTROL

*Attempts to Control Mites on Established Plants.*—The mites penetrate deeply into the crowns of the plants and are there protected from insecticides. Several attempts to control the mites on the established plants in the field were unsuccessful. In this connection calcium cyanide dust, nicotine dust, Selocide<sup>a</sup> spray, and nicotine spray were used. Naphthalene and carbon disulfide were tested, as fumigants, on plants under bell jars, but proved highly injurious to the plants. None of these materials proved to be of value.

Spraying established plants with lime-sulfur, and dusting with sulfur, were tested by Massee (1933) without appreciable success. An extended series of field tests were performed by Savzdarg (1928) who tested 22 different sprays including soap, oil, nicotine, quassia, sodium arsenite, iron sulfate, and sulfuric acid. None of these materials gave satisfactory results. He further performed 55 tests with dusts and fumigants including calcium cyanide, sulfur, naphthalene, paradichlorobenzene, and tobacco dust. These materials either failed to kill the mites, or else killed the plants. All of these tests indicate that field control on established plants is exceedingly difficult.

*Attempts to Control Mites on Planting Stock.*—Since the control of this pest on established plants seems impracticable, in the light of present knowledge, the remaining alternative consists in establishing fields free from mites. This can be done by planting clean plants in clean soil, sufficiently far away from other patches to prevent reinfestation.

The present conditions of strawberry culture in California lend themselves readily to this procedure. Planting stock is produced in the northern part of the state and in Oregon and Washington. (Klondike plants for southern California are grown in the East.) Hence, large shipments of packaged plants are concentrated in the hands of nurserymen and may be easily treated by a short stop-over en route or at final destination. New plants are always planted in soil which has not been cropped to

<sup>a</sup> Potassium ammonium seleno-sulfide.

strawberries for many years. At the present time, in central California a planting generally bears fruit for two years, and is then abandoned. Hence, under this system new plants are shipped in, planted in new, clean ground, and the whole acreage is replaced every three years. The major problem, therefore, lies in securing mite-free planting stock.

This might be accomplished by producing plants on mite-free soil, from mite-free mother plants. This method, however, has not been tested, and it is open to criticism, since a light reinfestation, difficult to detect, might occur at any time. However, under certain conditions, it may be possible to establish and maintain mite-free plantings in isolated areas. In the commercial production of plants, newly developed daughter plants are planted in the spring. These become the mother plants of the new field. They produce many stolons and daughter plants during the first growing season. These plants are dug in the following spring, the mother plants are discarded, and the rest are marketed. Some of the most vigorous daughter plants are kept and planted to produce another crop of plants. Thus while the mother plants reach an age of two years, the remainder of the plants are removed when one year old. Because of this rapid turnover of plants, the mites do not have an opportunity to increase in numbers, as is the case in fields devoted to fruit production, where the plants are generally four years old when discarded. Although mites have been found in plant-producing fields it is generally difficult to discover them. Hence, inspection of a plant-producing field in order to certify the plants as free from mites is impossible.

The most feasible procedure, therefore, apparently lies in the disinfection of planting stock. The first attempts to disinfect planting stock were conducted with fumigants in a tight chamber. A series of tests using cyanide, carbon disulfide, nicotine, and paradichlorobenzene were performed at atmospheric pressure and outdoor temperature. Results showed that these materials produced serious plant injury at dosages necessary to produce a complete kill of mites.

*Early Tests of Hot-Water Immersion of Planting Stock.*—During the winters of 1930–31 and 1931–32 a series of tests was conducted in which plants were immersed in hot water, in an attempt to determine a temperature and length of time of immersion which would prove lethal to the mites and yet noninjurious to the plants. In this work it was found necessary to use old, heavily infested plants for mite-control tests, since mites do not occur in sufficient numbers on young planting stock. On the other hand, it was necessary to use young planting stock for the plant-injury tests since old mother plants, especially when heavily infested with mites, are not properly indicative of the heat injury that would be suffered by young plants.

The plants were treated in a small vat made of two-inch redwood, containing 45 gallons of water. The water was heated by a 5,000-watt electric heater. When the atmospheric temperature was 61° F the heater raised the temperature of the water at the rate of 75° per hour. A verti-

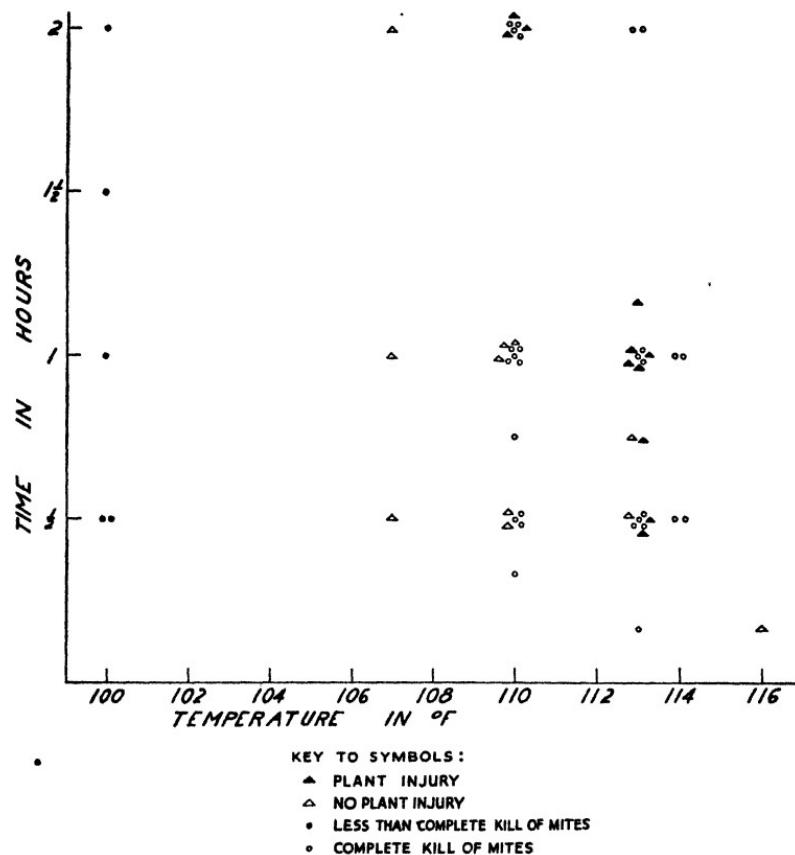


Fig. 5.—Results of hot-water immersion tests conducted in 1930-32.

cal agitator shaft provided with four propeller blades, each 3 by 4 inches was turned at the rate of 145 revolutions per minute in a direction to force the water up along the shaft. The plants were suspended in the tank in a basket of quarter-inch hardware cloth. When the temperature of the water had reached the desired point, two-thirds of the heating unit was disconnected. The rate of heating was then about the same as the rate of cooling. The temperature was thermostatically controlled, and checked with a three-inch immersion thermometer calibrated in

0.1° C. In the earlier work, the temperature of the water varied  $\pm$  1.0° F but in later work the range was  $\pm$  0.4° F.

The results of tests conducted during the winters of 1930-31 and 1931-32 are presented in figure 5. In this chart each mark indicates an independent treatment. The results show that a complete kill may be obtained by as short a period as 20 minutes at a temperature of 110° F and that no apparent plant injury was detected when the plants were immersed for as long as one hour at 110°.<sup>7</sup> As a consequence, subsequent work was centered around a temperature of 110° F for periods varying from 20 minutes to 2 hours.

*Hot-Water Immersion Tests Conducted in 1932-33.*—In the winter of 1932-33 two series of hot-water treatments were carried out in which lots of 15 plants each were immersed at temperatures of 109°, 110°, and 111° F for periods of  $\frac{1}{2}$ , 1, 1½, and 2 hours. The tests were duplicated with young infested planting stock, and heavily infested two-year-old plants. After treatment, the plants were held indoors, in moss in pots. Approximately one month after treatment each plant was carefully dissected under a binocular microscope. A few plants in the half-hour and one-hour treatments were found to support a single mite each, while a very few plants supported several mites each. The absence of eggs and larvae indicated that no breeding had occurred although the plants had been in a warm room for about a month. It was discovered later that the survivors of the heat treatment were specimens of another species of *Tarsonemus*<sup>8</sup> which was found in great numbers on raspberries. The strawberry plants used in these tests were obtained from a commercial field contiguous to a large planting of raspberries. Raspberry canes, bearing specimens of the new species of *Tarsonemus* were subjected to hot-water immersion. The results of this test, shown in table 5, indicate that this species is much more resistant to heat treatment than *T. pallidus* and that an appreciable percentage may survive an hour at 110° F.

The presence of this new species on strawberry plants is probably accidental. No cases of injury produced by this mite have been discovered. Floyd Smith<sup>9</sup> states "The species apparently does not cause primary injury as does *pallidus* and until this status changes we may ignore its presence from an economic standpoint."

In addition to the above-mentioned tests on mite control a series of treatments was conducted during the winter of 1932-33 to yield further information on the question of plant injury. The factors which varied

<sup>7</sup> Later work indicated that under certain conditions injury may be produced by this treatment; see discussion of 1934 results.

<sup>8</sup> Identified by Floyd Smith and E. H. Ewing of the United States Department of Agriculture Bureau of Entomology, as an undescribed species.

<sup>9</sup> Personal communication.

in these tests were (1) temperature of the water, (2) length of time of immersion, (3) time of year of treatment, and (4) variety of strawberry. The temperatures used were 109°, 110°, and 111° F. The periods of immersion were  $\frac{1}{2}$ , 1,  $1\frac{1}{2}$ , and 2 hours. The treatments were conducted on January 1 and February 5. The varieties were Nich Ohmer, Blakemore, and Z-54 (a hybrid between Fendalcino and Marshall; Fendalcino is derived in part from wild *chiloensis* parentage). Ten, or in some cases 5, young runner plants were used in each lot and every possible combination of the above factors was tested, except that Z-54

TABLE 5  
RESULTS OF HOT-WATER IMMERSION OF RASPBERRY CANES  
AT 110° F TO KILL AN UNIDENTIFIED SPECIES  
OF TARSONEMUS\*

Immersion, minutes	Number alive	Number dead	Per cent killed
20	54	1	1.9
30	16	12	42.8
40	64	11	14.7
50	15	12	44.4
60	7	31	81.6

\* Identified by Floyd Smith and E. H. Ewing of the United States Department of Agriculture Bureau of Entomology, as an undescribed species.

was not treated on January 1, and two time intervals were omitted. Checks of 10 untreated plants of each variety were planted on the same dates as the treated plants. In all, plants of 52 different treatments were planted.

Observations on March 10 on the February treatment indicated no differences in growth resulting from the variation in temperature. Nich Ohmer appeared the most tolerant to heat treatment and Z-54 was the most susceptible to injury. In the case of Nich Ohmer, plants of the half-hour and one-hour treatments grew more vigorously than the check. Definite injury appeared only in the two-hour treatments. In the case of Blakemore, injury appeared in the  $1\frac{1}{2}$  hour treatments and in Z-54, injury appeared in the one-hour treatments.

All of the plants were dug during the last few days of March. The number of flowers and young fruits were recorded as well as the weights of the plants.

Observations on the growing plants and counts at the time of digging indicate that the flower buds were much more susceptible to hot-water injury than other parts of the plant. In the commercial strawberry fields, flowers are removed by hand from the plants during the first year, so that strong vegetative growth may occur. Hence, hot-water injury to flower buds is not detrimental from the economic standpoint.

When all the treatments (except Z-54) at various periods, for various lengths of time, were summarized on temperatures, the average number of flowers and fruits per ten plants was 79 for the 109° F treatments, 78 for 110° F, and 31 for 111° F treatments; while untreated plants produced 125 flowers and fruits. This relation to temperature was not

TABLE 6

PERCENTAGE REDUCTION OBTAINED BY IMMERSION AT 110° F FOR FOUR TIME  
INTERVALS, DATA ARRANGED TO CONTRAST DATES OF TREATMENT

Variety	Weight of plants		Number of fruits	
	Jan. 1	Feb. 5	Jan. 1	Feb. 5
Nich Ohmer.....	2.2	-1.9	78	68
Blakemore.....	0.2	-0.3	67	86
Average.....	1.8	-1.1	73	77

TABLE 7

INFLUENCE OF LENGTH OF TIME OF IMMERSION AT 110° F,  
AVERAGE PER TEN PLANTS

Period of immersion. hours	Weight of plants, grams	Number of fruits
½.....	153	125
1.....	125	60
1½.....	114	16
2.....	118	14
Control.....	119	137

indicated by the weights of the plants. The average weight per 10 plants was 119 grams for the 109° F treatment, 123 for the 110° treatment and 128 for the 111° F treatment. The check plants weighed 119 grams. In table 6 and 7, values obtained for each temperature were averaged and assigned to the mean temperature of 110° F. For a comparison of the two dates of treatment, the data have been computed in terms of percentage reduction from the check values, and grouped on time of treatment in table 6. For a comparison of the length of time of treatment, the data have been regrouped and presented in table 7.

*Effect of Heat Treatment on Mite Eggs, 1933-34.*—During the winter of 1933-34, which, as stated above, was an unusually warm winter, the mites continued to deposit eggs. This raised a question as to the efficacy of hot-water immersion for killing the eggs of the mite. A series of treatments with 10 plants per lot was conducted on February 19, at 110° F for periods of 20, 40, 60, and 80 minutes. After treatment, the plants were potted in moss, and kept indoors. On March 12 and 29 the plants

were dissected under a binocular microscope. The plants of all treatments were found to be free from mites. Since these plants carried many eggs at the time of treatment it appears that a treatment as short as 20 minutes at 110° F will give complete control of all stages.

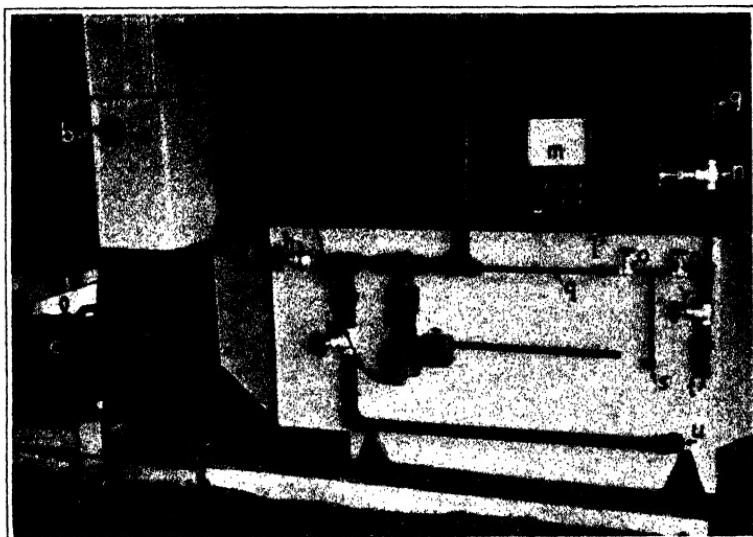


Fig. 6.—Air conditioning unit of the vapor apparatus: *a*, air duct from blower to vapor room; *b*, wet and dry-bulb thermometers for determination of relative humidity; *c*, motor for driving the blower; *d*, blower; *e*, armored cable lead from sensitive-bulb unit located in air duct *a*, which acts thermostatically on automatic steam valve *j*; *f*, main steam line from boiler to air conditioner; *g*, main cold-water line to air conditioner; *h*, steam valve to radiator unit, the opening of which gives a dry heat, which reduces the relative humidity as the air blast passes through and around the radiator; *i*, manually controlled steam valve which is auxiliary to automatic steam valve *j*; *j*, thermostatically controlled steam valve; *k*, sliding weight, the action of which works directly on automatic valve *j*, and thus provides for entrance of more or less live steam as desired; *l*, sensitive-bulb unit (several of which are buried in the strawberry plants at various locations in the vapor room), in which the resistance, recorded at the galvanometer *m*, varies directly with the heat in the room and, by converting ohms resistance to degrees Fahrenheit, makes it possible to obtain the temperature of any location in the vapor room at any time; *m*, galvanometer unit; *n*, main cold-water valve; *o*, auxiliary steam valve for combination steam and water jet, the entrance to the conditioner of which is *s*; *p*, auxiliary cold-water valve for combination steam and water jet, entering at *s*; *q*, auxiliary steam line for entrance of combination jet at *s*; *r*, cold-water valve for entrance to main water jet at *t*; *s*, entrance to auxiliary combination steam and cold-water jet; *t*, entrance to main cold-water jet; *u*, entrance to main live steam jet. (Photograph by California State Department of Agriculture.)

Infested planting stock was treated on February 2, 1934, at a temperature of 110° F for periods of 20, 30, 50, and 80 minutes. One hundred plants were used in each lot. After treatment, the plants were potted in soil and kept outdoors. On May 4, symptoms of mite injury appeared on

70.3 per cent of the check plants and no symptoms appeared on any of the treated lots.

**Hot-Vapor Treatments.**—A series of tests was conducted in an attempt to control the mites by heat applied to the plants by hot air and water

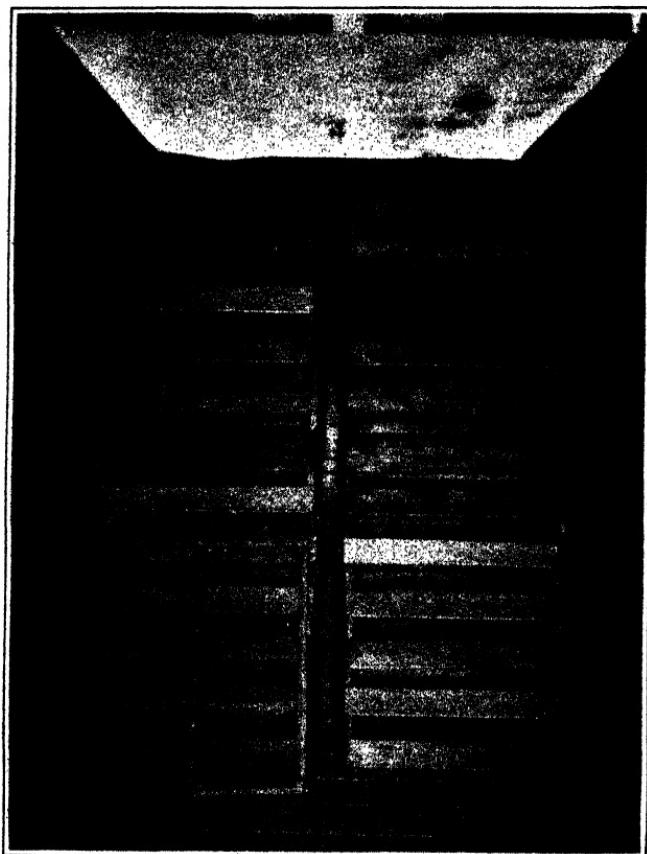


Fig. 7.—Treating room of the vapor apparatus, showing type of trays used and their arrangement in the room.

vapor. An apparatus (figs. 6 and 7) described by Mackie (1931) was used. Either 10 or 20 old, heavily infested plants were used in each lot. The relative humidity was 99 per cent. Plants were subjected to a temperature of 114° F. for 25 minutes, 45 minutes, 1½ hours, 2 hours, and 3 hours; and at a temperature of 110° F. for 45 minutes, 1½ hours, 2 hours, and 3 hours. These treatments were conducted on March 5, 1934. After treatment the plants were held in boxes in moss, outdoors. The plants were dissected under a microscope on March 14 to 28. Of the ten

plants treated for 25 minutes at 114° F, 8 were free from living mites, although several hundred dead adults and eggs were found on each. One plant carried one living adult female, and one had several living females and eggs. None of the plants in any of the other treatments contained living mites.

*Varietal Tolerance to Heat Treatment.*—During the winter of 1933-34, ten varieties and selections, listed in table 8, were treated by immersion in water at 110° F for one hour. Time and temperature were constant throughout, while each of the ten varieties was treated on February 4 and 20, and on March 3 and 18. Ten or 15 plants were used in each lot. The inclusion of the varietal factor in this experiment had its origin in a treatment in 1932. The limitations of hot-water immersion for strawberry plants were not well known at this time; but since it was desirable to replant certain varieties and selections held for observation and breeding work, without transferring mites, about 20 different lots were immersed in hot water. It is believed that certain controllable factors may have contributed to the rather severe root injury that resulted generally; but regardless of these factors, it was observed that certain varieties such as Heflin and Warfield showed but little injury. On the other hand, three pure *chiloensis* strains were nearly or entirely killed.

The choice of a group for this experiment covering different varietal reactions was to some extent influenced by the availability of plant material. It was thought that possibly varietal tolerance might be to some extent related to the following characters: early as compared to late-fruiting varieties, fleshy versus fibrous-root types, and selections carrying recently introduced *Fragaria chiloensis* strain. Three out of the ten lots used are commercial varieties; the remaining ones are from seven clones selected from crosses made in connection with the strawberry-breeding work of the Division of Plant Pathology of the University of California. The identification numbers and letters of the selections mentioned herein are those used by the Division and as such are a part of the permanent record for this project. Four of these selections contain some *F. chiloensis*, namely: J-31 (Marshall × Ettersburg 614<sup>10</sup>), 36.4 (Kalicene × Howard 17, synonym Premier), 37.2 (Kalicene × Missionary), and 99.2, a selection from a cross between descendants of Marshall × Ettersburg varieties. Selections 7.18 and 7.26 were derived from a cross between Nich Ohmer and Blakemore, while 103.3 is from Nich Ohmer by AO-8, the latter a seedling of U.S.D.A. 253.<sup>11</sup>

<sup>10</sup> The varieties, Ettersburg 614 and Kalicene were originated by Mr. A. F. Etter of Ettersburg, California, who has been crossing named varieties with various strains of *F. chiloensis* for a number of years.

<sup>11</sup> Field record number of the United States Department of Agriculture strawberry-breeding project.

TABLE 8

VARIETAL TOLERANCE TO TREATMENT; PLANTS TREATED ON FEBRUARY 4 AND 20  
AND ON MARCH 3 AND 18

Variety	Percentage reduction in weight			
	Flowers and fruit, March 29	Flowers and fruit, June 2	Plants	Average
Klondike.....	13.3	-3.1	4.4	4.9
J-81.....	29.4	58.1	-37.6	16.6
Nich Ohmer.....	41.1	26.2	5.6	24.3
103.3.....	28.9	33.9	18.9	27.2
7.18.....	55.6	29.5	4.6	29.9
Dorsett.....	65.9	60.4	7.1	44.4
7.26.....	64.7	57.8	10.9	44.5
99.2.....	55.1	63.1	20.7	46.3
37.2.....	70.0	72.2	18.4	53.5
36.4.....	85.4	62.8	21.4	56.5

TABLE 9

INFLUENCE OF TIME OF TREATMENT UPON PLANT GROWTH

Treated	Percentage reduction in weight			
	Flowers and fruit, March 29	Flowers and fruit, June 2	Plants	Average
February 4.....	42.8	50.0	9.9	34.2
February 20.....	76.2	68.2	15.1	53.2
March 3.....	34.0	21.5	15.4	23.6
March 18.....	.....	24.0	4.6	14.3

TABLE 10

RESULTS IN PLANT GROWTH FROM IMMERSION OF NICH OHMER PLANTING STOCK  
AT 110° F.; TREATED FEBRUARY 2

Immersion in minutes	Weight of flowers and fruit, grams			Weight of runners, grams
	March 30	May 4	Total	
None (check).....	127.0	307.8	434.8	5.7
20.....	220.0	342.8	562.8	14.7
30.....	222.0	327.5	549.5	18.0
50.....	212.2	320.8	533.0	21.4
80.....	187.3	343.9	531.2	29.4

After treatment, all lots were planted outdoors in a mixture of sand, peat, and manure. A lot of untreated plants of each variety, equal in number to the treated lot, was planted on each of the four dates of treatment. Each individual lot of treated plants was planted in a row beside the row of check plants. The number of check plants was therefore equal to the number of treated plants. On March 29 all flowers and fruits together with fruiting stalks were picked and weighed. On June 2, all plants were dug, the weights of the new crop of reproductive structures were taken, as well as the total weight of the plants, after the reproductive structures were removed.

When the weight of the treated plants was subtracted from the weight of the untreated plants, the difference was believed to be the result of treatment. This difference has been computed into per cent of the weight of the untreated plants as presented in table 8. A minus quantity indicates a gain.

When the data on these ten varieties and selections are averaged to show the influence of time of treatment the results presented in table 9 are obtained.

On February 2, 1934, locally grown Nich Ohmer planting stock was immersed in water at 110° F for periods of 20, 30, 50, and 80 minutes. One hundred plants were used in each lot. After treatment plants were potted in soil, outdoors, and moved into a greenhouse on March 10. On March 30, all flowers and fruit were removed and weighed; on May 4 a second crop of reproductive structures was removed and weighed, as well as all runners. The data so obtained are given in table 10. In this test, it was found that the weight of reproductive structures decreased slowly with an increase in length of immersion, but the plants of all treatments surpassed the untreated. Runner production increased with an increase in length of immersion.

*Comparison of Water-Immersion with Water-Vapor Treatments.*—A test to compare immersion with water-vapor treatments at 110° F for plant injury was conducted on March 1, 1934. In this test, northern-grown Nich Ohmer planting stock was used, approximately 20 plants in a lot. Vapor treatments were conducted at a humidity of 95 per cent and at 99 per cent each for periods of  $\frac{3}{4}$ ,  $1\frac{1}{4}$ , 2, and 3 hours. Hot-water immersion tests were performed simultaneously at the same temperature (110° F) for periods of  $\frac{3}{4}$  and  $1\frac{1}{4}$  hours. All plants, together with an untreated check, were planted in a commercial field immediately after treatment.

On June 2 the mortality of the plants and the number of runners produced were recorded as given in table 11. This table indicates that by June 2 only minor differences in growth were noted. However, the fact

that runner production was greater in every treated lot than in the check is of considerable importance from the commercial viewpoint.

A test of varietal tolerance to vapor treatment and to hot-water immersion was conducted on March 2. In this test five varieties were treated at 110° F for 1½ hours each in vapor and water immersion. Ten plants were used in each lot. After treatment, all plants, together with untreated checks, were set outdoors in a mixture of sand, peat, and manure.

TABLE 11  
COMPARISON OF IMMERSION AND VAPOR TREATMENTS AT 110° F,  
ON NICH OHMER PLANTS

Treatment	Time treated	Plants alive	Plants dead	Number of runners per 20 plants
Immersion.....	45 minutes	20	0	51.0
	75 minutes	18	2	49.0
Vapor, humidity 99 per cent.....	45 minutes	19	0	54.8
	75 minutes	18	1	76.6
	2 hours	16	0	66.2
	3 hours	17	3	62.6
Vapor, humidity 95 per cent.....	45 minutes	18	1	52.2
	75 minutes	18	0	62.2
	2 hours	19	0	64.2
	3 hours	37	1	63.8
Untreated.....	.....	25	0	49.6

On April 16 all flowers, fruits and fruiting stalks were picked and weighed. On June 1 the plants were dug and the weights of reproductive structures and of the plants, recorded. The weights of reproductive structures obtained on April 16 and June 1 were combined. The difference between the treated and untreated plants was computed as per cent reduction from the untreated condition. These data are presented in table 12. A negative reduction represents a gain in weight.

*Influence of Storage of Plants in Relation to Treatment.*—Several tests were conducted to determine the influence of storage on plants, before and after treatment. These tests were duplicated with the varieties Blakemore and U.S. 542. All treatments were hot-water immersion for one hour at 110° F. Twenty plants were used in each lot. The temperatures of storage were constant at about 34° F in some cases, and storage outdoors in normal February weather in other cases. Plants were stored before and after treatment for three weeks at 34° F. Checks consisted of plants treated without storage, and stored without treatment. Plants were held at outdoor temperatures for three weeks, then

treated and planted. Plants treated in the above-stated manner were nearly uniform in growth on June 2. The plants which had been held in storage at 34° F for two weeks following treatment were slightly more vigorous than those of the other treatments and checks.

TABLE 12

COMPARISON OF WATER-IMMERSION AND VAPOUR TREATMENTS OF STRAWBERRY PLANTS AT 110° F FOR 1½ HOURS COMPUTED AS PERCENTAGE REDUCTION FROM WEIGHTS OF CHECK PLANTS

Variety	Treatment	Flowers and fruit	Runners	Plants	Total
Nich Ohmer.....	Immersion.....	6.8	.....	-23.0	-16.2
	Vapor.....	-160.7	.....	-43.9	-204.6
Dorsett.....	Immersion .....	94.6	-89.2	-21.5	- 16.1
	Vapor.....	71.4	-91.4	-20.9	- 40.9
58.5.....	Immersion.....	36.8	30.0	21.1	87.9
	Vapor.....	-160.2	62.4	-15.6	-113.4
66.6.....	Immersion.....	90.8	.....	-53.2	37.6
	Vapor.....	- 10.2	.....	-61.2	- 71.4
103.2.....	Immersion.....	62.8	.....	24.6	87.4
	Vapor.....	52.7	.....	23.3	76.0
Average all varieties.....	Immersion.....	58.3	-11.8	-10.4	36.1
	Vapor.....	- 41.4	- 5.8	-23.7	- 70.9

TABLE 13

INFLUENCE OF HOT-WATER IMMERSION ON WEIGHTS OF REPRODUCTIVE STRUCTURES OF NICH OHMER PLANTS

Length of time immersed	Temper- ature, degrees Fahr.	Time (hours) × tem- perature (degrees Fahr.)	Weight of flowers fruit, grams	Length of time immersed	Temper- ature, degrees Fahr.	Time (hours) × tem- perature (degrees Fahr.)	Weight of flowers fruit, grams
2 hours.....	100	218	3.6	45 minutes.....	110	83	41.6
1½ hours.....	110	165	5.7	30 minutes.....	111	56	47.8
1½ hours.....	100	164	22.6	30 minutes.....	110	55	76.0
1 hour.....	111	111	24.2	30 minutes.....	109	54	65.0
1 hour.....	110	110	15.7	20 minutes.....	111	37	104.3
1 hour.....	109	109	54.2	20 minutes.....	110	36	78.8

*Further Tests of Immersion Conducted in 1934-35.*—During the winter of 1934-35, mite-control tests conducted earlier were repeated. Two-year-old Nich Ohmer plants were obtained which (by microscopic examination after treatment) were found to contain from 300 to 500 adult females each. Fifteen plants were used in each lot. Treatments for 20, 30, and 45 minutes were given by hot-water immersion at 110° F. After

treatment the plants were carefully dissected under a microscope and no living mites were found.

An attempt was made to secure data on mite control and plant injury by treating Nich Ohmer planting stock which had been infested by hand transfers of mites in August, 1934. The plants were dug and treated in lots of 40 plants each at temperatures of 109°, 110°, and 111° F for

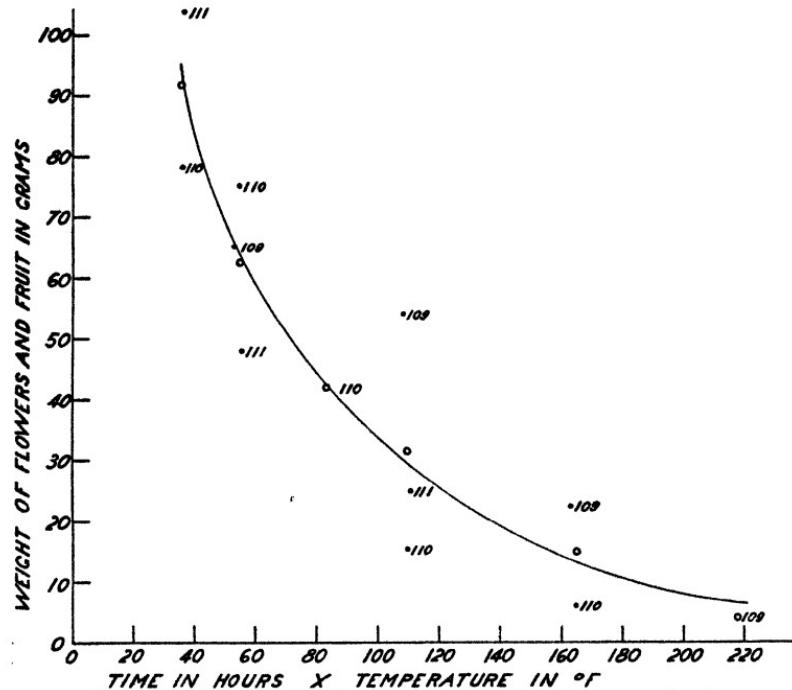


Fig. 8.—Relation of weight of reproductive structures produced, to time and temperature of immersion.

periods of 20, 30, 45, 60, 90, and 120 minutes. One hundred untreated plants constituted a check. After treatment all lots were planted in soil boxes in a heated greenhouse. The check plants were all found to be infested with mites and no mites appeared on any of the treated plants. At the time the first fruits were coloring, all fruits, flowers, and fruiting stalks were picked and weighed (table 13). Signs of hot-water injury appeared on the leaves of the plants treated at 109° F for 1 hour, as well as on those of the higher temperatures and longer treatments, but were absent on those subjected to the lower temperatures. When the weights of fruit obtained at the different temperatures were averaged on each time interval, and plotted against time in hours multiplied by temperature in degrees Fahrenheit, the curve plotted in figure 8 was obtained.

## DISCUSSION AND CONCLUSIONS

The success of the control measures herein recommended depends upon the thoroughness with which mites are killed on the planting stock, and the thoroughness of the precautions taken to prevent reinfestation of the plants in the field. In view of the large number of plants listed as hosts of the mite, it might seem impossible to establish and maintain a field sufficiently remote from all of these plants to prevent contamination. However, most of the plants listed are attacked only in greenhouses, and of the remainder, which are attacked out of doors, only a very few, if any, are capable of carrying the mites through the winter. Under out-door California conditions, the authors to date have not found *Tarsonemus pallidus* occurring naturally on any plant other than strawberry.

Varieties of strawberries exhibit varying degrees of susceptibility to mite injury. However, the Marshall (Banner) variety is the only commercial variety grown in central California which generally escapes injury of economic proportions. The variety Klondike, while severely injured in the central coastal section is seldom severely injured in southern California. Hence, Marshall and Klondike will not, under ordinary conditions, require treatment. All other varieties, and particularly Nich Ohmer, should be treated.

After the plants have been disinfested by heat treatment, *utmost precautions must be exercised to prevent reinfestation*. Boxes of treated plants must not be stacked near untreated plants; treated plants must not be repacked in unsterilized boxes, and in unsterilized moss, etc. The most important measure to prevent reinfestation consists in locating new plantings sufficiently distant from old heavily infested plantings. The rapidity with which mites are spread from old infested fields to new uninfested fields is indicated above. Wherever possible new disinfested plantings should be at least 500 yards from all old infested areas.

Under the present practice, workmen who plant, weed, cultivate, and irrigate a new planting, alternate such labor with a few days of berry picking in old established fields. *Such movements of laborers from old to new patches is a dangerous potential source of reinfestation*, and should be accompanied by all possible precautions, such as washing the hands, changing clothing, or not entering the new patch on the same day as the old.

The only satisfactory method found for killing all mites on planting stock consists in heating the plants. Two methods of accomplishing this result have been studied namely hot-water immersion and hot-vapor treatment. In the immersion method, 20 minutes at 110° F kills all

mites; but in order to insure a complete kill under all conditions the treatment should be continued for half an hour. In the hot-vapor method a complete kill is obtained in 45 minutes at 110° F, but, as an additional precaution, this treatment should be continued for one hour.

The findings presented in this paper are supported by the results of other workers, since heat treatments of strawberry runners for the control of *Tarsonemus pallidus* have been studied in other parts of the world. The first recommendation of a hot water dip was that of H. Garman in 1884 (Munger, 1933), who stated that infested verbenas were freed from mites by dipping in water at 120° F for half a minute. Hodson (1933) in England, was apparently the first to apply heat treatment to strawberry runners. After four years of investigation he concluded that immersion for 20 or 30 minutes at a temperature of 110° F was the most desirable treatment. Hot-water immersion of strawberry plants was studied in Canada by Dustan and Matthewman (1932) who obtained favorable results. This method was further tested in England by Massee (1934) who concluded that 20 minutes immersion at 110° F was preferable. Doucette and Baker (1932) treated plants in a vapor apparatus at 110° F for periods of 1½ and 3 hours and considered that the method could be perfected to be of economic value. Floyd Smith (1933) tested vapor treatments on greenhouse plants and concluded that 30 minutes exposure to vapor at 110° F was as effective as 15 minutes immersion in water at the same temperature.

While either vapor treatments or hot-water immersion may be used satisfactorily, the authors prefer the vapor treatment, because the plants are more easily handled and respond better in growth. Plants removed from a vapor room are nearly dry, and as they cool rapidly (in 45 to 60 minutes) they can soon be repacked for shipping. In contrast, plants removed from a dipping vat are saturated with water, and must be dried before they can be repacked. This requires considerable space under roof, as they must be protected from direct sunlight and excessive air movement. When large numbers of plants are treated by this method, it is necessary to turn them, to facilitate even drying. All of this is laborious, expensive, and requires unusual facilities. Inherent in such a situation will be a tendency to hasten some phase of the process, resulting in either allowing the surface plants to become too dry or packing the plants too wet. This latter may involve serious losses because of the activity of saprophytic organisms, particularly if it is not possible to plant immediately.

In addition to the advantages existing in the mechanics of plant management for the vapor method, the plants themselves show less injury, and grow better, as is indicated in table 12. This comparative response

is corroborated by field experience. In the spring of 1934 a strawberry grower treated about 200,000 Nich Ohmer plants in hot water, and another lot of 200,000 plants in a vapor apparatus. These plants were set out in alternating small patches in the same field. During the first month the vapor-treated plants excelled the water-immersion plants, but by September no differences could be seen.

Plants destined to be treated by either method should be given every possible protection from drying. Observations indicate that any appreciable drying of the plants renders them more susceptible to heat injury; also the added exposure to the air, incident to treatment, may cause some dehydration of the roots, which is injurious in itself. If, in the use of the vapor method particularly, plants must be held in the trays two or three hours prior to treating, they should be covered with a canvas. If the roots are very dry or the weather is unfavorable, some method of lightly sprinkling them probably should be devised. After they have cooled following treatment the same protection should be given. The practice of some plant growers of sprinkling their fields before digging, if soil moisture or temperature are unfavorable, is a further protection, in addition to the suggestions given above. One other safeguard needs mentioning here. If, at planting time, the soil is dry or the weather very warm, irrigation should be given immediately.

As mentioned in the reference to commercial use of heat treatments for mite control, heat-injured or retarded plants have pronounced recuperative powers. In general, the data herein presented were taken while the effects were still measurable, but not as visibly distinct as earlier. Thus, notes taken February 28, 23 days after treatment, indicate definite retardation and twisting of the leaves in some varieties listed in table 8; but by June 2, when the final readings were taken, these symptoms had disappeared. On this date some varieties in this group still showed a 20 per cent reduction in weight. This loss would in all probability have been overcome by the end of the growing season.

For the purpose of discussion, plant injury is divided roughly into four distinct categories. Probably the most common one is temporary inhibition of the growth processes. This is not necessarily a concomitant of the control measure, for the authors have records of definite stimulation. Results to date do not indicate that this form of injury is of any practical consequence. Next in the order of discussion is what has been termed "crown injury." It is characterized by retardation and production of small, twisted leaves. It usually disappears in a few weeks. It is not expected that this form of injury will be encountered in the present commercial varieties used in the central district and treated at the time-temperature interval that is herein recommended. The third type of

damage is the killing of the foliage, indicated by a brown or blackened appearance of the leaves the day after treatment. It occurs only when plants which have become rather active in the spring, are treated, and then mostly in the longer time intervals used in the experiments. In spite of their poor appearance such plants usually grow, but the presence of this injury in commercial practice should mark the seasonal limits for any particular year. The last category to be considered is that of root injury. It can easily be recognized by the dark, water-soaked appearance of the cortex and may affect only a few or, in some cases, all the roots. Usually the degree of injury is progressively less from the root tips toward the crown, often ending before reaching that point. Generally the stele in the basal part of the root will not be damaged and will initiate new growth. The strawberry-breeding project with which the junior author is associated has afforded an excellent opportunity for observation of this phenomenon. In one case 900 different selections were treated for mite control. Unexpectedly, severe root injury ensued, entailing, it was thought, serious losses. As a matter of fact, only 10 or 12 plants out of approximately 1,800 died, and the entire planting made very good growth. Subsequently, many of these same selections have been given much more severe treatment, without any appreciable amount of this type of reaction. The factors conditioning this form of injury are not well understood. Degree of dormancy, low temperature, and heavy frosts when plants are dug and treated are suggested as possibly having some relation to injury. In some cases it is apparently definitely related to varietal tolerance, of which more will be said later. Any cortical lesions or discoloration of the stele existing in the roots prior to treatment may result in killing of the roots, with symptoms somewhat similar to those mentioned above; but such killing should not be attributed primarily to the treatment. Some surface discoloration of the roots often occurs, particularly in immersion-treated plants. It is caused chiefly by prolonged exposure to the air and is in no wise injurious.

The studies in plant tolerance, relative to certain of its conditioning factors herein reported are in their implications no wise final; it is believed, however, that some generalizations and practical applications can be derived from their consideration.

Experience showed that the reproductive structures were much more sensitive to heat than the vegetative structures. As an example of this, in table 12, the variety Dorsett shows a difference of 23.2 per cent in favor of vapor treatment, in the reproductive structures, in contrast to only 0.6 per cent in favor of vapor in the vegetative structures. Because of this sensitivity the weight of the reproductive structures served as a useful index to the various factors studied.

In general this index, supplemented by weight of plants, has been found of value, particularly with reference to time and temperature, methods of heat application, and varietal tolerance. No satisfactory data have been obtained for the best season to apply treatment. It is difficult to arrange any set of experiments designed to study this factor that are directly comparable.

In general, and contrary to expectation, the supposedly fully dormant plant seems to be the more susceptible to heat injury. In one experiment it was evident that plants treated on February 5 made far better growth than those treated on January 1. It was obvious that this advantage was not wholly a function of the treatment, because the untreated plants for the respective dates reflected the same relative values. Apparently much of this retardation was the result of moving and resetting plants during the dormant or rest period. This and other observations seem to imply an optimum season for the digging and treating of plants, with reference to favorable plant response. From the information now available, and because no identical calendar dates will be comparable for any consecutive period of years, no fixed dates can be given for this optimum season. For the region in which these investigations were conducted, the latter part of February and most of March is suggested.

Time of treatment is undoubtedly of some importance, but regardless of the time of year which seems most desirable, it will be necessary to treat when weather conditions permit the digging of planting stock, and the setting of plants in the field. Further, it must be adapted to the practices proven best by experience for any specific region. Thus in the district to which these studies are pertinent, plants are usually set in March and April, after winter rains have ceased. Because of the recuperative powers of the strawberry, it is recognized that from the practical viewpoint the end result, in terms of mite control, final costs, vigor, and stand of the planting at the close of the growing season, is the only adequate measure of this or any other phase of the treatment for mite control.

Earlier in the discussion the relation of variety to heat injury was mentioned. The wide range of response of several varieties to both methods of treatment are given in tables 8 and 12. The relative resistance of these varieties as indicated in table 8 cannot be expected to maintain the same sequence under all conditions of treatment. Replication of this test would probably show some shifts in position. Different stages of bud differentiation in the several varieties and selections may account in part for the varying degrees of injury to these parts; but regardless of such considerations the conclusion can be drawn that great differences as to heat tolerance exist in strawberry varieties, and that certain varie-

ties such as Klondike, J-31, and Nich Ohmer are relatively resistant. Further, that some varieties such as Dorsett are relatively susceptible, as has been substantiated by field experience, and that selections 36.4 and 37.2 are very susceptible. The latter selections have for one of their parents Kalicene. This fact serves to introduce the subject of recent incorporation of *Fragaria chiloensis* in strawberry varieties in its relation to heat tolerance. Susceptibility to injury has been frequently observed in such selections, but sufficient exceptions have been noted, such as J-31, to make any generalization relative to them unwarranted. In recent treatments of breeding stock it was noted that some selections from certain crosses showed considerable root injury, whereas all selections from other crosses showed practically none. So far as is known no recent *F. chiloensis* is involved in this case. Summing up, it may be said that to date no type of plant or character of growth could be definitely associated with heat injury in the varieties studied. In the use of any varieties not previously tested, it is suggested that preliminary trials be made to determine their specific reaction.

In the spring of 1934, 6,000 plants were treated for one-half hour at 110° F and distributed to growers in lots of 1,000 plants each. In two cases the plants were held in packing boxes for two or three weeks after treatment and a high mortality of plants occurred. In the tests of storage described above, made before and after treatment, there was no air conditioning in the cold-storage room used, so that the plants tended to be dehydrated. Some plants were held for six weeks and apart from this drying no unfavorable results were observed. The use of a room in which the humidity can be kept relatively high and constant should largely obviate this difficulty. By repacking the contents of one planting box into two or three boxes, alternating a layer of plants with a layer of moist moss, it should be possible to hold plants in storage satisfactorily for a considerable period of time. The best practice, of course, is to treat the plants as soon as possible after digging, and to plant as soon as possible after treatment.

In the experiments reported in this paper, considerable work has been carried on in the longer time intervals. By this means it has been possible to study more satisfactorily certain factors involved in heat injury. Further, outside of a temporary retardation, the time-temperature interval of one hour at 110° F is within the practical limits for Nich Ohmer under most conditions. As shown in the data (table 7), definite injury generally occurs in the hour-and-a-half, and two-hour intervals, and is usually directly observable in the form of "crown injury." Root damage does not necessarily occur with this type of injury, so that under experimental conditions such plants usually grow. Tests show that consider-

ably longer time intervals may be used in the vapor method before comparable injury occurs.

## SUMMARY

The cyclamen mite, *Tarsonemus pallidus* Banks, incorrectly known as the strawberry mite, *Tarsonemus fragariae* Zimm., is a widely distributed pest of field and green house strawberries. Since its first appearance on strawberries in 1892 it has been reported in 10 European countries and 27 states in the United States.

The mite causes severe injury to Nich Ohmer strawberries in central California, while the Marshall variety is resistant in this area. The Klondike variety is not seriously affected in southern California.

The majority of adult mites are females, which lay two or three eggs a day. The immature stages are completed in about 13 days, hence a very rapid increase in population is possible.

The mites live in the folded leaves in the center of the crown of the plant. Their feeding punctures cause severe distortion and dwarfing of the leaves.

Only adult females survive the winter. They hibernate in the crowns between the bases of the petioles. They emerge from hibernation about the latter part of February.

No satisfactory method of controlling the mites on established plants has been found. However, they can be eradicated from planting stock and the field subsequently kept free from mites.

A complete kill of mites on planting stock can be obtained by immersing the plants in water at 110° F for 30 minutes, or by treating the plants with saturated air at 110° F for one hour. Of these two methods, the vapor treatment is superior to the immersion method.

For treatment, plants must be removed from shipping boxes, and loosened if tied in bundles. They should be placed loosely in screen or slat trays or boxes and stacked to allow a maximum of penetration of either the hot water or vapor. After treatment the plants should be cooled and dried before repacking. Precautions should be taken to prevent excessive drying. If plants are repacked while wet, saprophytic fungi may kill a large percentage of them.

Varieties show varying sensitivity to heat treatments. Klondike and Nich Ohmer are relatively resistant while Dorsett is more easily injured.

In either method the temperature must be accurately held at 110° F and should not vary more than one degree. The heating medium (water or vapor) must be thoroughly agitated to insure uniform exposure of all plants to the desired temperature.

Planting should follow treating as soon as possible. Plants should be set in clean soil as far from old infested fields as possible, preferably 500 yards or more.

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# H I L G A R D I A

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## EFFECT OF OIL SPRAY ON CALIFORNIA RED SCALE AT VARIOUS STAGES OF DEVELOPMENT<sup>1,2</sup>

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### INTRODUCTION

DURING SEVERAL YEARS of field investigation with oil sprays used against the California red scale, *Aonidiella aurantii* (Mask.), the writer has observed that often some insects may not have received a sufficient amount of oil to cause sudden death and that they may live for two or three weeks after other individuals have been killed. Where the lethal action is thus prolonged, the typical signs of death, such as dryness or discoloration of the body fluids, may not be definitely discernible until six weeks or more after the spray treatment. In many cases examinations of infested citrus groves a month after treatment with oil spray have revealed an unsatisfactory degree of control of the red scale; at the end of six weeks, however, the mortality of the insects was very much higher.

The work of deOng, Knight, and Chamberlin (1927, p. 372) indicated that the lethal effect of highly refined oil sprays is the result of suffocation of the insect, but Smith (1932) has observed that often the oil does not reach the tracheae, and in that case death may be caused by a "prolonged impairment of physiological processes such as might be induced by the presence of oil in the scale covering or in contact with the derm of the insect's body." In cases in which the lethal effect is retarded because of incomplete suffocation, the appearance of the insect indicates that it has some oil in its body. Its scale covering is oily, and a certain amount of dust has adhered to the oil film.

Another factor enhancing the effectiveness of oil sprays beyond their

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initial lethal capacity is believed to be the inhibition of the settling of the scale crawlers<sup>4</sup> for a period after the application of an oil spray and the high rate of mortality among those which are able to settle. Woglum (1926) states that the residual oil prevents the settling of crawlers "for a considerable period" on the fruit and new wood, and Woglum and La Follette (1934) more recently conclude that the inhibition of the settling of the young insects in this manner is "one of the most important considerations in red-scale control by oil spray." Since the mortality resulting from oil sprays is known to be sometimes very low, especially on lemon trees and on rough bark (Ebeling, 1932), the inhibition of the settling of the crawlers born of the insects surviving the spray treatment may be a salient factor to consider in an appraisal of the ultimate efficiency of oil sprays.

Other factors, aside from the obvious factor of tracheal penetration, that might influence the effectiveness of an oil spray treatment, such as suffocation caused by sealing of the edges of the scale by the oil, penetration of oil through the derm of the insect, and the effect of oil on reproductivity, were deemed worthy of experimental investigation in order that a more complete understanding might be obtained concerning the nature of the reaction of red scale to oil spray. This knowledge, in turn, might serve as a basis for experimentation on control measures. In order that the work might be systematically pursued, the reaction of each life stage of the red scale to oil spray was considered separately.

#### INHIBITION OF THE SETTLING OF CRAWLERS

Experiments were made to determine to what extent the settling of red-scale crawlers is inhibited by oils of various degrees of heaviness and sprays at different concentrations. On October 16, 1932, two leaves of an orange seedling were treated with a grade-4 (medium)<sup>5</sup> oil applied at 2 per cent concentration as a tank-mixture spray (Smith, 1933) by means of a quart-capacity hand sprayer; the remainder of the seedling was left untreated. On October 17, 20 red-scale crawlers were placed on each of 2 sprayed and 2 unsprayed leaves. A camel's-hair brush was used to transfer the crawlers. The petioles of the leaves were banded with tanglefoot so as to keep the transferred crawlers from leaving the leaf. Two days later the leaves were examined so that the whitecaps,<sup>6</sup> if any, could be counted and removed; 20 more crawlers were then placed

<sup>4</sup>"Crawlers" are the newly born young of the red scale; they move about for a period of several hours to two days, after which they settle on the host and, in the case of the females, remain sessile throughout their lives.

<sup>5</sup> Distillation range: 10 per cent at 592° F and 90 per cent at 722° F.

<sup>6</sup> After the crawlers have settled, they secrete a white, waxy substance which covers their bodies and gives them the common designation "whitecaps."

on each leaf. This process was repeated every two days until December 10. The seedling was kept in a laboratory at a mean temperature of 65° F.

No whitecaps occurred on the sprayed leaves until November 2, 16 days after the application of the spray. On that date 1 whitecap was found on leaf A. On leaf B, no whitecap appeared until November 16,



Fig. 1.—Residual effect of oil sprays on two twigs of an orange seedling. Left, twig untreated; right, twig sprayed with 2 per cent grade-4 (medium) oil. Over a period of 53 days after treatment, 400 red-scale crawlers were placed on each twig. Note defoliation of unsprayed twig.

30 days after the application of the spray. The reason for the great difference in the dates of the first appearance of crawlers on the two sprayed leaves is not apparent, unless it can be accounted for by unintentional variation in the application of the spray, resulting in a greater deposit of oil on leaf B. Only 3 whitecaps had appeared on leaf A, however, before the first whitecap appeared on leaf B. After November 16 the number of crawlers able to settle on the sprayed leaves gradually increased until on December 8, 11 settled on leaf A and 9 on leaf B. During all of this time the number settling on the unsprayed leaves varied between 5 and 17 with an average of 10 per observation on each leaf.

In another experiment two twigs of a lemon seedling were progressively infested with red-scale crawlers. One of the twigs had been sprayed with 2 per cent medium oil and one had been left untreated as a check. Twenty crawlers were transferred to each twig every second day for 40 days. The sprayed twig was isolated by a band of tanglefoot. The spraying was done on October 26, and on December 3 the first white-

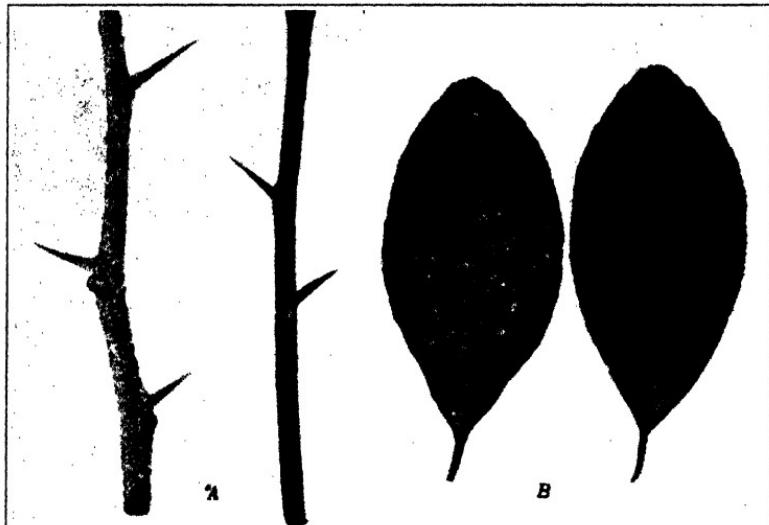


Fig. 2.—Residual effect of oil spray on an orange seedling. *A*, Portions of the two twigs shown in figure 1. Note the large numbers of scale, especially whitecaps, on unsprayed twig (left) and absence of scale on the sprayed twig (right), caused by failure of crawlers to become established after spray treatment. *B*, Leaf taken from unsprayed twig (left) and leaf taken from sprayed twig (right) of orange seedling shown in figure 1. Note absence of scale on sprayed leaf.

cap was found on the twig which had been treated. Thus 39 days had elapsed before a single crawler was able to settle on the sprayed twig.

Figures 1 and 2 are photographs which were taken on January 26, 1933, three months after the first crawlers had been transferred to the lemon seedling. On 1 inch of the unsprayed twig 3 mature females, 6 males, and 112 whitecaps were counted. The sprayed twig was entirely free of insects, the one crawler that settled on December 3 having died. The large number of whitecaps and of red scales in other stages on the unsprayed leaf (fig. 2, *B*) illustrates the rapidity with which a dense population of red scale is built up; it also indicates that the inhibition of the settling of crawlers may be a potent factor in minimizing the seasonal population of the red scale.

*Effect of Heaviness of Oil.*—Having experimentally established the

fact that the presence of oil on the surface of the citrus tree inhibits the settling and subsequent development of the crawlers, it was considered desirable to determine the differences in the inhibitory effects of sprays composed of oils of different degrees of heaviness. The sprays were tank mixtures and included two grades of spray oil: grade-5 (heavy) oil,<sup>7</sup> used commercially only in the spraying of lemon trees; and a grade known commercially as Oronite Crystal,<sup>8</sup> which is heavier than any oil used in citrus spraying.

A vigorous two-year-old orange seedling was selected for the purpose of this experiment. On December 20, 1932, one leaf was sprayed with 2 per cent Oronite Crystal oil, an adjacent leaf was sprayed with 2 per cent grade-5 (heavy) oil, and a third leaf was left untreated as a check. The spray was applied, as in previous experiments, with a quart-capacity hand sprayer. The petioles of the leaves were banded with tanglefoot. The tree was left in a well-lighted room at a constant temperature of 80° F.

From December 29, 1932, to February 28, 1933, 25 red-scale crawlers were transferred to each leaf every 2 days; the numbers of whitecaps found were recorded and the whitecaps were then removed.

No whitecaps were found on the leaf sprayed with grade-5 oil until January 20, 1933, 31 days after the spray was applied. On the leaf sprayed with Oronite Crystal oil, the first whitecap was found on January 26, 36 days after the treatment. By February 28, there were 14 whitecaps on the leaf sprayed with grade-5 oil, and only 1 on the leaf sprayed with Oronite Crystal oil: 333, or an average of 15.13 whitecaps per day, had been found on the check leaf.

*Effect of Oil on Whitecaps.*—A condition that would so profoundly affect the settling and development of the crawlers might be expected to affect also the future development of those individuals which are able to settle and reach the whitecap stage. Experiments were made to determine to what extent, if any, the presence of oil on citrus foliage would hinder the development of whitecaps.

On March 20, 1933, red-scale crawlers were placed on each of the leaves referred to above. Ninety days after the leaves had been treated, 100 crawlers were placed on the leaf sprayed with Oronite Crystal oil (leaf A), 300 on the leaf sprayed with grade-5 (heavy) oil (leaf B), and 100 on the check leaf (leaf C). Two days later 1 whitecap was found on leaf A, 31 on leaf B, and 70 on leaf C. On April 6 the leaves were examined again to determine the percentage of whitecaps which had survived on the three leaves. The single whitecap on leaf A had died, 16 had

<sup>7</sup> Distillation range: 10 per cent at 612° F and 90 per cent at 737° F.

<sup>8</sup> Distillation range not known; viscosity 120 seconds Saybolt.

survived on leaf B, and 58 had survived on leaf C. The percentage of survival, therefore, was as follows: leaf A, 0.00 per cent; leaf B, 51.61 per cent; and leaf C, 82.83 per cent. On another leaf, treated on January 7 with a spray containing grade-5 oil at 2 per cent concentration, and upon which 300 crawlers had been placed on March 20, 5 whitecaps out of 17 survived, making a survival of 29.41 per cent.

*Experiments Made under Commercial Conditions.*—Observations made in groves which had been sprayed for red scale indicated that the

TABLE 1

## NUMBER OF RED-SCALE WHITECAPS FORMING ON SPRAYED AND UNTREATED ORANGE TWIGS\*

Treatment	Number of whitecaps		Crawlers developing into whitecaps, per cent
	Leaf A	Leaf B	
Fifty crawlers transferred March 3, 1933			
Grade 4 oil (medium) $1\frac{2}{3}$ per cent .....	1	8	9
Grade 1 oil (light) $1\frac{2}{3}$ per cent.....	19	23	42
Check.....	32	36	68
Fifty crawlers transferred March 10, 1933			
Grade 4 oil (medium) $1\frac{2}{3}$ per cent .....	14	10	24
Grade 1 oil (light) $1\frac{2}{3}$ per cent.....	23	27	50
Check.....	31	39	70

\* Sprayed by means of a power sprayer, Feb. 10, 1933.

period of inhibition of the settling of crawlers caused by the presence of oil spray on the foliage was shorter in commercially sprayed trees than on the seedlings which had been experimented with in the laboratory. This was probably caused mainly by the difference in the nature of the spray deposit between foliage sprayed with a power sprayer and that sprayed with a hand sprayer. Although the foliage of the seedlings treated in the laboratory did not receive sufficient oil to cause any appreciable signs of injury, such as dropping of the leaves, it had probably received a heavier deposit of oil than if the spray had been applied with a power sprayer. Furthermore, the dark green, vigorous leaves found on rapidly growing seedlings do not allow as rapid penetration of oil as the average leaves on mature orchard trees. Factors peculiar to outdoor conditions, such as direct sunlight and air movement, no doubt also had their effect in shortening the period of inhibition.

On February 10, 1933, a number of large Washington Navel orange trees were sprayed with  $1\frac{2}{3}$  per cent grade-4 (medium) oil and  $1\frac{2}{3}$  per

cent grade-1 (light) oil with spray prepared by the tank-mixture method. The spray was applied with a power sprayer, No. 7 disks being used in the nozzles; a pressure of 400 pounds was employed. On March 3, twigs were taken from trees sprayed with the two grades of oil, as well as from a check tree, and were placed in jars of water in a room at 80° F constant temperature. Two leaves from each twig were selected, to which were transferred red-scale crawlers from heavily infested lemons. Fifty

TABLE 2

## THE EFFECT OF OIL SPRAY IN INHIBITING THE SETTLING OF RED-SCALE CRAWLERS AND THE DEVELOPMENT OF WHITECAPS\*

Date sprayed	Date crawlers were transferred	Seedling No.	Insects on sprayed seedling		Insects on check seedling	
			First instar, examined April 28, 1933	Second instar, examined May 9, 1933	First instar, examined April 28, 1933	Second instar, examined May 9, 1933
April 22, 1933	April 26, 1933	1	4	1	40	37
		2	4	0	37	33
		3	0	0	28	27
		4	12	2	34	29
		5	1	0	24	20
		6	5	1	29	26
		Av.	4.3	0.7	32.0	28.7
April 22, 1933	May 12, 1933	1	12	6	15	11
		2	11	8	18	16
		3	9	4	29	26
		4	20	8	33	28
		5	19	15	25	24
		6	6	3	26	23
		Av.	12.8	7.3	24.3	21.3

\* Sprayed by means of a power sprayer.

crawlers were placed on each leaf, and the petioles of the leaves were banded with tanglefoot. Transfers were made in a similar manner on March 10. The results of the experiment are shown in table 1.

Of the crawlers transferred to the sprayed leaf on March 3, 21 days after the spray treatment, only 9 per cent formed whitecaps on the two leaves sprayed with 1½ per cent medium oil as compared with 68 per cent on the two check leaves. Seven days later the percentage on the sprayed leaves had increased to 24. This, however, indicates a marked inhibition of the settling of crawlers even a month after the spray is applied. If the crawlers had been transferred outdoors instead of in the laboratory, the percentage forming whitecaps would have been very small, and comparisons between treatments would have been less significant.

On April 22, 1933, 6 orange seedlings were sprayed with a grade-5 (heavy) oil at ¾ of 1 per cent concentration, the equipment employed

being the same as that of the previous experiment. The seedlings were placed in a room at 80° F constant temperature. Four days later one leaf was chosen from each of the sprayed seedlings and one from each of 6 seedlings left unsprayed as a check. Fifty crawlers were placed on each leaf. On April 28, counts were made of the number of whitecaps on the sprayed and check leaves, and on May 9 counts were made of the number of insects which continued development to the second instar. On May 12, a leaf on each of the seedlings sprayed April 22 and one on

TABLE 3

## EFFECT OF GRADE-5 (HEAVY) OIL IN INHIBITING THE SETTLING AND DEVELOPMENT OF RED SCALE UNDER ORCHARD CONDITIONS

Date of transfer, 1933*	Crawlers forming whitecaps		Whitecaps reaching second instar†	
	Sprayed tree‡	Unsprayed tree	Sprayed tree‡	Unsprayed tree
August 11.....	0	33	..	..
August 18.....	2	60	..	..
August 25.....	15	51	..	..
September 1.....	23	90	3	60

\* A total of 200 crawlers transferred on each date to each tree, with 50 crawlers on each of four leaves.

† Records kept only for crawlers transferred September 1 and forming whitecaps.

‡ Sprayed August 4, 1933.

each of the check seedlings was again chosen for infestation. Fifty crawlers were placed on each leaf, and observations were made as in the previous experiment. The data regarding the two experiments are presented in table 2.

Although only  $\frac{3}{4}$  of 1 per cent grade-5 oil was used in the spray mixture, only 8.6 per cent of the crawlers transferred to the seedlings 4 days after the spraying developed into whitecaps as compared with 64.0 per cent on the check seedlings. When the crawlers were transferred 20 days after spraying, 25.6 per cent developed into whitecaps as compared with 48.6 per cent on the check seedlings.

*Observations on Crawlers Transferred under Orchard Conditions.*—The purpose of this experiment was to determine the behavior of crawlers transferred in the orchard, where the mortality is higher than in the laboratory, probably mainly because of low night temperatures. On August 4, 1933, a lemon tree was sprayed with grade-5 oil at  $1\frac{2}{3}$  per cent concentration and an adjacent tree was left unsprayed as a check. The  $1\frac{2}{3}$  per cent concentration is the usual dosage used for red scale on lemons. At intervals of one week, 50 red-scale crawlers were transferred from heavily infested lemons to each of 4 leaves on both the sprayed and check trees. Each time crawlers were placed on the leaves, those settling from previous transfers were removed. As in previous experiments, the

petioles of the leaves were smeared with tanglefoot and the crawlers were transferred by means of a camel's-hair brush. The results of the experiment are given in table 3.

Of the crawlers transferred to the sprayed leaves 28 days after the application of the spray 11.5 per cent formed whitecaps, but only 1.5 per cent survived to the second instar. On the check leaves 45.0 per cent formed whitecaps and 30.0 per cent survived to the second instar.

TABLE 4

## OILINESS OF RED-SCALE CRAWLERS AT VARIOUS INTERVALS AFTER TREATMENT\*

Date, January, 1933	Number of crawlers	Number of crawlers oily in less than 30 seconds	Number of crawlers oily in less than $\frac{1}{2}$ hour
16.....	20	20	20
17.....	20	20	20
18.....	20	20	20
21.....	20	8	20
22.....	10	3	10
23.....	20	4	18
26.....	20	4	11
27.....	20	2	8
30.....	10	0	0

\* The crawlers were placed on orange leaves sprayed with 2 per cent grade-5 (heavy) oil on January 15, 1933.

*Behavior of Crawlers after Spray Treatment.*—In an effort to determine the cause of the inability of crawlers to settle on oil-sprayed leaves, a potted lemon seedling was sprayed with 2 per cent grade-5 (heavy) oil. At various periods thereafter for two weeks, lots of 10 or 20 crawlers were transferred to certain leaves of the sprayed seedling, and the leaves were then removed for examination with a binocular. The results of the experiment are shown in table 4.

The crawlers placed on the seedling the first day after the latter was sprayed, were immediately mired in the oil on the surface of the leaf and could not move. They were soon covered with a film of oil. Those placed on the seedling during the next two days could move some distance with difficulty, but the margin of the body would often come in contact with the oil on the leaf surface, and because the waxy surface of the body of a crawler is wet so readily, a film of oil crept over the body of each insect, usually within 10 or 15 minutes. The creeping of oil over the body of a crawler may be watched with the aid of a binocular. The crawlers are yellowish and opaque when placed on the sprayed leaf. After moving about for some time they gradually become oily and translucent, and for the first day or two after the leaf is sprayed they may actually draw a pool of oil from the leaf surface about their bodies.

Of the 20 crawlers placed on the seedling on the sixth day after spraying, only 8 were covered with an oil film from the leaf surface within 30 seconds, and the majority were able to crawl about for half an hour before becoming oily. Among those placed on the seedling on the eighth day, 4 out of 20 were affected in this manner within 30 seconds and 18 out of 20 before the end of a half-hour period. Among the crawlers placed on the seedling on the fifteenth day, none were appreciably covered with oil even after dying and having a large portion of their bodies in contact with the leaf surface. None, however, were able to form whitecaps. After the oil film had for the most part penetrated into the leaf, the crawlers would often live long enough to insert their beaks into the leaf, but would die before reaching the whitecap stage.

Comparative studies on the inhibition of the settling of red-scale crawlers were not made on the rough bark because of the great variability in the capacity of the bark to absorb oil and because of the very small percentage of crawlers able to settle even on the unsprayed bark. Owing to the fact that the oil film is less persistent on the bark than on the leaf surface, the settling of crawlers may be inhibited for a much shorter period.

*Attempts at Control Directed against Crawlers.*--The practicality of killing or inhibiting the development of crawlers by repeated spray treatments with small concentrations of oil is very remote. This is owing to the large number of applications necessary over perhaps a two-month period in order to effect a control. The frequency of application is made necessary by the rapid absorption of the oil by the bark, on which the crawlers are often very numerous.

Experiments were made to investigate the possibility of inhibiting the development of crawlers by the use of various dusts, such as lime, talc, diatomaceous earth, and sulfur, with or without a certain amount of oil or toxic substance mixed with the dust. In the laboratory citrus seedlings could be kept free from red-scale infestation by dusting them with sufficient quantities of an oil-impregnated or even an undiluted, inert dust. In these experiments thousands of red-scale crawlers were transferred to treated and untreated seedlings and the number settling on the leaves and twigs was determined. A 100 per cent inhibition of settling was often obtained. Field experiments, however, showed the impracticability of this type of control on a commercial basis, because of the impossibility of applying dust in sufficient quantities on both sides of the leaves all through the trees and because of the large number of treatments necessary.

## EFFECT OF OIL SPRAY ON IMMATURE STAGES

*Crawler Stage.*—Since the crawler and whitecap stages are killed by even such small amounts of oil as are found on or in the leaves several weeks after spraying, obviously they must succumb to the oil actually sprayed upon them, even at the lowest concentrations used in citrus spraying.

TABLE 5

DIFFERENCE IN SURVIVAL OF ADULT AND IMMATURE RED SCALE

Life stages	Per cent of oil	Treated insects		Check (untreated) insects		Net mortality, <sup>1</sup> per cent
		Total	Per cent alive	Total	Per cent alive	
Adults . . . . .	{ 1	1,776	25.9	1,437	84.4	69.3±0.80†
	2	1,576	15.4	1,437	84.4	81.8±0.71
Immature stages‡	{ 1	1,861	3.6	1,472	84.6	95.7±0.34
	2	1,567	1.3	1,472	84.6	98.5±0.23

\* Calculated as  $\frac{x-y}{x}$  when  $x$  = percentage left alive in untreated lot and  $y$  = percentage left alive in treated lot.

† Probable error computed from the formula  $P.E. = 0.6745 \sqrt{\frac{pq}{(n-d)}}$  when  $p$  = net per cent mortality;  $q = 100 - p$ ;  $n$  = number of insects counted; and  $d$  = number of  $n$  which would have died from natural causes during the course of the experiment as determined by the percentage of dead insects in the check. The P.E. is less reliable for extremely large values of  $p$  or  $q$ , as with the data under "Immature stages" in this table, because deviation from the mean becomes less symmetrical as  $p$  and  $q$  vary from the condition in which  $p=q=50$  per cent.

‡ Immature stages include those from the first molt to and including the gray adult. Grade-5 (heavy) oil was sprayed January 14, 1935, and the insects examined February 1, 1935.

*Other Immature Stages.*—All other immature stages of the red scale (fig. 3) are likewise killed by far less oil than is necessary to kill the adults. Thus, a 1 per cent concentration of grade-5 (heavy) oil sprayed on the fruit, green twigs, or leaves of the lemon, or a 1 per cent concentration of grade-3 or 4 (light-medium or medium) oil on the fruit, green twigs, or leaves of the orange, is sufficient to kill nearly all immature stages of the red scale up to and including the "gray adult." Some may be left alive, however, when the same amount of oil is sprayed on the bark.

To secure more data regarding the matter, lemons heavily infested with red scale were obtained, and the insects showing by their appearance that they were dead were removed. All fruits were then sprayed with grade-5 oil at 1 per cent and 2 per cent concentrations by means of a laboratory precision sprayer. The fruit was kept in paraffin paper sacks in a room at a temperature of approximately 65° F. Eighteen days after treatment the number of live adults and the number of live immature stages from the first molt to and including the gray adult were

<sup>1</sup> By "gray adult" is meant the stage between the second molt and the mature adult.

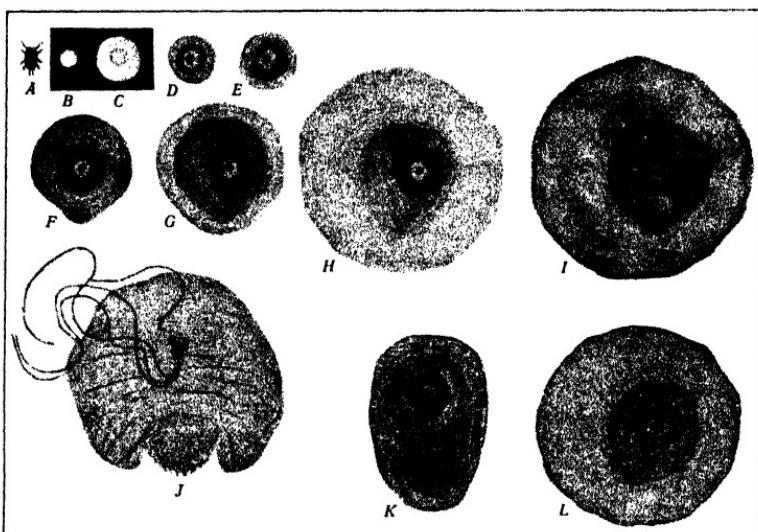


Fig. 3.—The development of the red scale as it appears on the plant. *A* to *J*. Stages of the female scale: *A*, motile young or "crawler"; *B*, white cap; *C*, nipple stage; *D*, first molt; *E*, secretion of gray wax margin after first molt; *F*, second molt; *G*, secretion of gray wax margin after second molt; *H*, completion of extension of scale covering by enlargement of gray wax margin. This stage may be called the gray adult. *I*, The adult or young-producing stage.

The first cast skin is incorporated in the scale covering, as shown by the darker inner circles in *F*, *G*, *H*, and *I*. The second cast skin is represented by the outer margin in *F*, and the same cast skin may be distinguished in *G*, *H*, and *I*.

In stages *D*, *F*, and *I*, the covering may not be lifted without taking with it the insect itself. In the other stages represented, the covering may be lifted free from the insect.

*J*, The adult female as it occurs under the scale covering *I*. The threads represent the mouth parts, which are inserted into the plant. Their length may be twice the diameter of the adult insect.

*K*, The scale covering of the male. The same first cast skin may be noted. The cast skins from the other three molts of the male are not incorporated in the scale covering. The winged male emerges from beneath the narrower (lower) end of this covering, at a time when the same-aged female is in stage *H*, or between stages *G* and *H*.

The photograph was taken from a colored chart and the different shades are not correctly reproduced. On the original chart, *L* represented the yellow scale. The yellow scale is similar in size to the red scale, but it is of a lighter color.

All of the figures are of the same relative size, with an enlargement of 20 diameters. (From Bulletin 542.)

determined. On the basis of counts made on control fruits the percentage of the insects that died from natural causes in the eighteen-day interim after the treatment was also determined. The results of the experiment are shown in table 5. The mortality of both the adult and the immature scales would have been much greater if the counts had been made a month or six weeks after treatment.

The ease with which scale in the immature stages may be killed with oil spray may be accounted for by the fact that, as compared with the adults, they are less firmly fixed to the surface upon which they rest and,

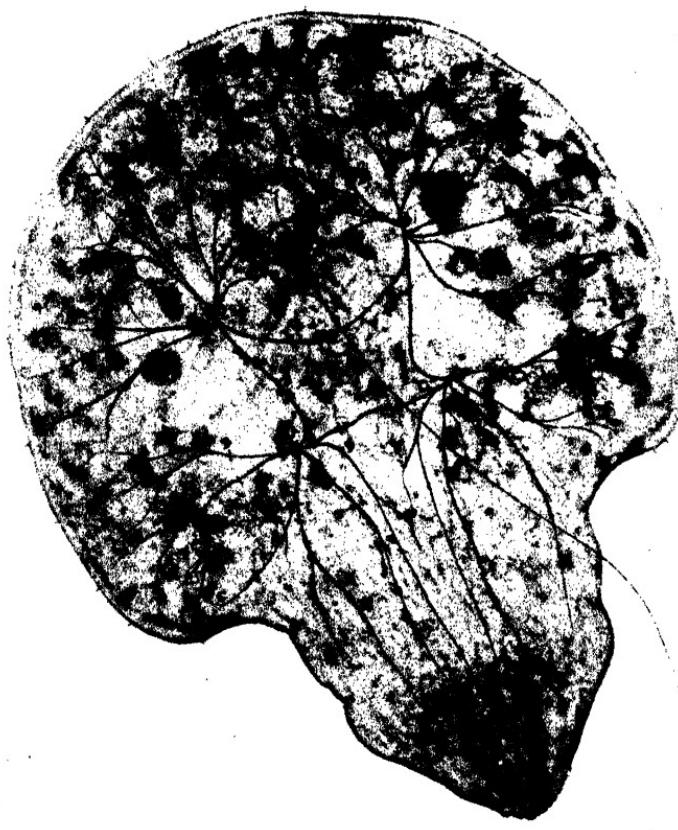


Fig. 4.—Ventral aspect of the California red scale, *Donidiella aurantii* (Maskell), showing the tracheal system. (From *Hilgardia* Vol. 2, No. 9.)

except during molts, are free from their armors. Also the spiracles of the immature insects are at a lesser distance from the margins of their bodies than are those of the adult scale; consequently less oil is necessary on the surface in order that some may reach the spiracles. The respiratory system of the female red scale in the gray adult stage is shown in figure 4. In this stage the armor may be lifted from the insect, and it is then that the red scale is an excellent subject for microscopic examination.

*Penetration of Oil into the Spiracles.*—An experiment was made in which lemons infested with red scale were sprayed with different concentrations of oil by means of a laboratory precision sprayer; the scales were examined at different intervals of time after the spraying to determine the percentage of insects having oil in their spiracles. Since insects of all stages were removed for microscopic examination from the same fruits, the usual variability in oil deposit had no influence on the results of the experiment.

TABLE 6

## THE PENETRATION OF OIL INTO THE SPIRACLES OF RED SCALE IN THE SECOND-MOLT, GRAY-ADULT, AND ADULT STAGES ON LEMON FRUITS

Percent of oil*	Hours after treat- ment	Second-molt insects			Gray-adult insects			Adult insects		
		Number with spir- acles visible	Number with spir- acles oily	Per cent with spir- acles oily	Number with spir- acles visible	Number with spir- acles oily	Per cent with spir- acles oily	Number with spir- acles visible	Number with spir- acles oily	Per cent with spir- acles oily
2%	3	25	22	88.0	23	19	82.6	21	6	28.6
2%	15	20	19	95.0	20	18	90.0	21	9	42.8
1	16	25	25	100.0	25	25	100.0	36	23	63.9

\* Grade-5 (heavy) oil sprayed.

† Separate records were made for insects not having all four spiracles visible; these are combined in this table for brevity.

The degree to which oil has penetrated the spiracles or tracheae can be easily discerned because of the change in refractive index of the contents of the tracheal tubes caused by the entrance of the oil. As deOng, Knight, and Chamberlin (1927) point out, "The low refractive index of the air-filled tracheae causes them to show as black lines under the microscope. If penetration of the liquid occurs, it causes an increase in the refractive index of the liquid-filled portion with a consequent lowered visibility, and the degree of penetration becomes plainly visible."

According to the results of these experiments, presented in table 6, a given amount of oil is more apt to reach and penetrate the spiracles of insects in the second-molt or gray-adult stages than those in the adult stage. Of the insects with spiracles that were visible with the aid of a microscope, 64.3 per cent of the adults had no oil in the spiracles when sprayed with an oil spray of  $\frac{2}{3}$  of 1 per cent concentration; 36.1 per cent had no oil in the spiracles when sprayed with an oil spray of 1 per cent concentration. The corresponding figures for the gray adult were, respectively, 14.0 per cent and 0.0 per cent; and for the second molt, 8.9 per cent and 0.0 per cent. Likewise, the spiracles were not visible on a greater percentage of the adults than of the younger stages, prob-

ably because no oil was present to make the wax covering the spiracles translucent. Only a small percentage of the spiracles of the adult insects are visible when the insects are not placed in a liquid capable of wetting the wax. The differences in the percentages of the spiracles reached by oil on the adults and on the younger insects are therefore actually greater than the data in table 6 indicate.

### EFFECT OF OIL SPRAY ON ADULT

*Structure of Adult.*—As compared with the immature stages of the insect, the adult red scale is rather invulnerable to oil. This is because of the structure of the adult and the tenacity with which it adheres to the surface upon which it rests.

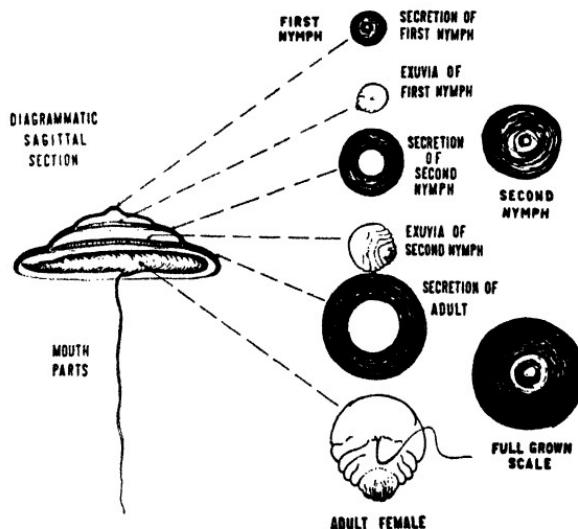


Fig. 5.—Structure and development of an armored scale.  
(Courtesy of Illinois State Academy of Science.)

According to Metcalf and Hockenyos (1930), the covering of the adult armored scale (fig. 5) consists of the secretion of the first-instar nymph and the skin shed by it, the secretion of the second-instar nymph and the skin shed by it, and the secretion of the adult female. In the case of the red scale, the ventral covering of the insect consists of the ventral cast skins or exuviae and a certain amount of wax. This covering is thin and pliable, however, compared with the hard, rigid dorsal armor of the insect (fig. 6).

The cross section of the insect (fig. 6) shows how well it is protected from insecticides by the unbroken covering extending both dorsally and

ventrally over its soft body. The dorsal covering or "armor" is the thicker, measuring about  $65\mu$  in thickness, while the ventral covering measures about  $20\mu$ . Both coverings appear solid and homogeneous in character under high magnification unless sections are cut very thin ( $2\mu$ ), in which case it appears that the coverings are composed of laminae with spaces between them. These spaces are discernible whether the scale has been prepared and stained for sectioning in the usual manner or merely embalmed in paraffin and sectioned without preparation of any kind.



Fig. 6.—Median cross section of the California red scale showing difference in thickness of the dorsal and ventral coverings of the insect ( $\times 145$ ).

The thin wax margin of the insect which is extended beyond its body after the molting period is composed of loosely woven threads of the wax; sometimes these may be seen exuding from glands on the pygidia of insects between molts. The waxy margin is, of course, very readily penetrated by oil, and this may be one of the reasons why the insects, when they are expanding under the waxy covering, are very vulnerable to oil spray.

The shed skins are composed of chitin, while the secretions are wax and a nonwaxy substance, the exact chemical nature of which, as regards the armored scales, is not known (Metcalf and Hockenjos, 1930). However, a very complete analysis of the secretions of a number of unarmored scales has recently been made by Kono (1932-35). The dried Florida red scale, *Chrysomphalus aonidum* L., was found by Metcalf and Hockenjos (1930) to consist of between 31 and 34 per cent of wax when carbon tetrachloride was used as the wax solvent. Haas (1934), using an ether extraction method, extracted wax averaging 10.17 per cent of the body dry weight of California red scale. These insects were taken from areas where they are known to be resistant to hydrocyanic acid gas fumigation, while from scale taken from nonresistant areas he ex-

tracted an average of 16.83 per cent of wax. The variation in the figures obtained by Metcalf and Hockenyos and by Haas on these closely related scales might be accounted for by the difference in the solvents used to extract the wax. Metcalf and Hockenyos (1930), after extracting 11 per cent of the total weight of oyster-shell scales with ether, extracted another 24 per cent with carbon tetrachloride. In the same paper these investigators state that the solubility of the wax of oyster-shell scales in grams per cubic centimeter at room temperature is 0.0008 gram with

TABLE 7

DIFFERENCES IN MORTALITY OF ADULT RED SCALES WITH AND WITHOUT THEIR ARMORS LIFTED ON ONE SIDE

Grade of oil used*	Date sprayed (1933)	Date examined (1933)	Treatment	Total sprayed	Alive, percent	Total check	Alive, percent	Net mortality, per cent†
3	July 18	Aug. 1	Lifted	1,470	28	1,090	64.8	95.7±0.44
			Unlifted	1,756	12.6	1,544	72.6	82.6±0.72
5	Aug. 2	Aug. 26	Lifted	368	10.8	266	71.0	84.8±1.50
			Unlifted	460	14.1	255	74.1	81.0±1.43
1	Oct. 4	Oct. 23	Lifted	3,562	19.7	2,138	79.6	75.3±0.55
			Unlifted	2,399	28.2	3,503	84.7	66.7±0.70
5	Oct. 4	Oct. 23	Lifted	2,078	10.4	2,138	79.6	86.9±0.58
			Unlifted	1,687	15.6	3,503	84.7	81.6±0.69

\* Oil used at 1½ per cent concentration in each test.

† Net mortality and probable error computed as in table 5.

carbon tetrachloride and 0.0084 gram with ether; hence, it would appear that ether is by far the better wax solvent and that extractions made with ether would be the more reliable. Carbon tetrachloride extracts a large number of organic compounds besides wax, and investigators generally have abandoned its use for the extraction of waxes and fats from organic material.

*Penetration of Oil into the Spiracles.*—It can be seen from table 6 that a much greater amount of oil is necessary to insure penetration into the spiracles of the adult scale insect than into those of the immature stages. As stated before, this is probably caused by the greater distance the oil must travel in order to reach the spiracles and the greater tenacity with which the adult insect is attached to its substratum. The adult, also, is supplied with a ventral covering, which probably aids in keeping oil away from the spiracles. This ventral covering is absent from immature insects between the molting periods.

If a sufficient amount of oil is deposited by the spray mixture, pene-

tration to the spiracles may occur in less than a minute. Since oil penetrates into solid wax very slowly, as can be readily demonstrated, the oil apparently must reach the spiracles by passing between layers and particles of wax and other substances comprising the bulk of the material lying between the body of the scale and its substratum.

A slight lifting or loosening of the margin of the scale over half of its circumference by means of a scalpel causes the oil to reach the spiracles very readily and greatly increases the mortality resulting from a given amount of oil spray, as is shown in table 7.

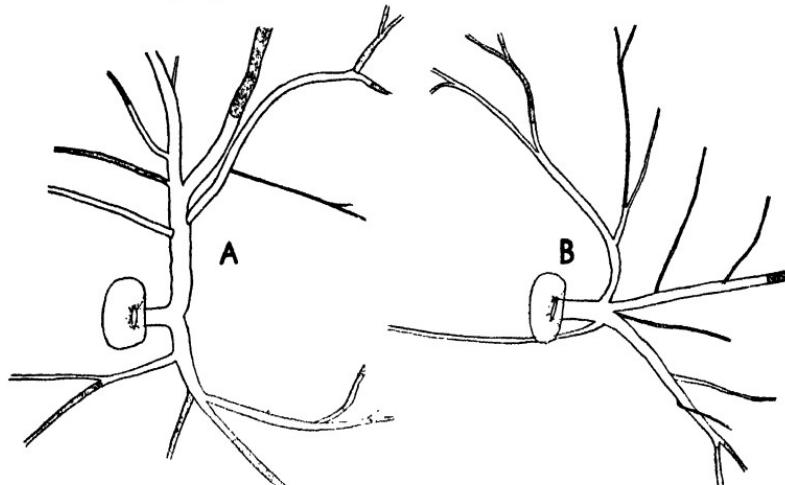


Fig. 7.—Main trunks of the tracheal system of the gray adult of the California red scale, showing the progress of oil into the tracheae. The stippled areas represent portions of the tracheae into which the oil has not yet penetrated. *A*, Left anterior spiracle; *B*, left posterior spiracle.

**Tracheal Penetration.**—Upon reaching the spiracles of a live insect, the oil immediately flows into the tracheae as if there existed no regulatory mechanism in the spiracle to exclude foreign matter even temporarily, although Nel (1933) suggests that ridge-like processes projecting into the lumen of the spiracle, together with certain muscles which he was able to observe, probably constitute a closing apparatus. The oil moves through the tracheae in a jerky manner, flowing rapidly for a distance, then ceasing movement for a short period. It may be that the periods between the movements of the oil indicate periods in which a certain amount of air already absorbed is being used by the insect. The air pressure within the trachea is thus gradually reduced until the inertia of the oil column is overcome by the capillary attraction of the oil to the wall of the trachea. There is a consequent sudden movement

forward of the oil column until it is again stopped by the pressure of unused air within the trachea.

Sometimes the oil will not enter some branches of the tracheae, although it enters others immediately and may reach the extremities of the tracheoles in a short time (fig. 7). The pressure set up in the tracheae by the inflow of oil may disrupt the ends of the tracheal branches, causing a rapid flow of oil without resistance from the air in the lumen of the

TABLE 8

RESULTS OF ATTEMPTS TO SUFFOCATE RED SCALE BY VARIOUS METHODS OF SEALING WITH BALSAM AND PARAFFIN WAX

Treatment	Number of insects examined	Net per cent mortality		
		Five days after treatment	Sixteen days after treatment	Thirty days after treatment
Margin smeared with balsam . . . . .	178	....	23.4	18.6
Margin smeared with balsam, armor smeared with oil . . . . .	246	95.1	95.2	100.0
Armor smeared with balsam, margin left free . . . . .	173	13.2	6.9	10.3
Entire insect covered with balsam . . . . .	208	51.8	93.8	94.0
Entire insect covered with paraffin . . . . .	180	73.0	82.9	100.0

tracheae. Many insects having oil in their tracheae also have one or more sharply defined bloated areas, indicating the presence of liquid within the body.

With regard to insects situated on smooth surfaces, such as the fruit, leaves, and green bark, a sufficient amount of oil is usually deposited by spray mixtures of average concentration to insure the penetration of oil into the tracheae in a very large percentage of the insects. Obviously these insects are killed by suffocation. Some, however, receive oil in only one, or possibly in none of the four spiracles. In these cases the oil entering the single spiracle, or the oil present in the body of the insect, may ultimately cause the death of the insect, although not until several weeks after the death of insects which had all the spiracles blocked with oil.

One might argue that even if the oil does not actually penetrate beneath the insect and enter the spiracles it may still suffocate the insect by sealing the edges of the armor. Attempts were made to suffocate red scales on lemon fruits by smearing the margins of their armors with balsam, the armors being left uncovered. Only a small number of the insects were killed, and these may have been broken loose from the substratum on which they were resting and been killed in this manner. As can be seen from table 8, the insect may obtain air either from beneath the margin of the armor or through the armor itself. That oil would

suffocate the red scale by sealing the margins of the armor, is therefore improbable, especially since the oil is for the most part drawn into the substratum on which the insect rests. Such absorption takes place within a period ranging from a few hours to one or two days, according to the nature of the substratum. Even when the entire insect is covered with

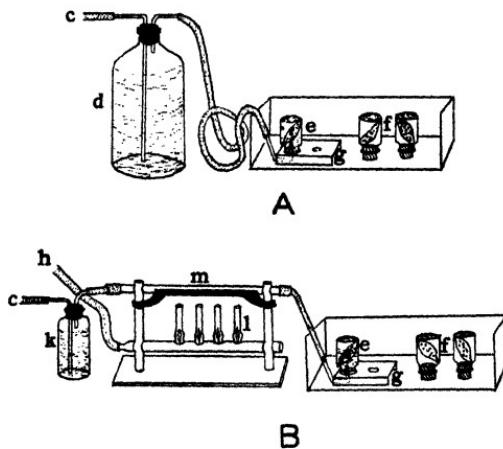


Fig. 8.—Experimental setup employed in removing oxygen from air and introducing the oxygen-free atmosphere in bottles containing red scale.  
*A*, Apparatus for pyrogallol method: *c*, tube leading to source of compressed air; *d*, bottle containing alkaline pyrogallol; *e*, bottle containing red scale on a lemon peel and water (the bottle is also immersed in water to above the neck); *f*, bottles having had the water within displaced by oxygen-free atmosphere; *g*, stand supporting bottle *e*. *B*, Apparatus for hot-copper method: *h*, tube leading to gas supply; *k*, bottle of concentrated sulfuric acid; *l*, gas burner; *m*, Pyrex tube containing copper turnings; others as in *A*.

balsam or paraffin, the percentage of mortality is not great for several weeks after treatment, the seal in this case probably being not entirely impervious to oxygen.

*Oxygen Requirements of the Red Scale.*—In view of the apparently small amounts of oxygen required by the red scale, it was considered advisable to determine how long the insect can live in an atmosphere as nearly as possible devoid of oxygen. Two methods were employed in freeing air of oxygen. Both are illustrated in figure 8. According to method A, air is slowly passed through alkaline pyrogallol prepared by combining 1 volume of 22 per cent pyrogallol with 6 volumes of 60 per cent potassium hydroxide. According to method B, air is slowly passed through concentrated sulfuric acid for dehydration, then over hot

copper turnings tightly packed in a Pyrex tube. In either case the air from which the oxygen has been removed is bubbled into wide-mouthed 8-ounce bottles (fig. 8, e) in which have been placed freshly cut lemon peelings heavily infested with red scale. The confinement of the scales in the bottles causes in itself no appreciable mortality. In these bottles the oxygen-free atmosphere displaces freshly boiled and cooled water.

When the bottles are full of the oxygen-free atmosphere, they are stoppered under water and left standing in water well over the neck of the bottle until the examination of the insects is made. In one instance red scales in bottles with oxygen-free atmosphere left standing in water without being stoppered were alive four days after treatment; others, in bottles of oxygen-free atmosphere that had been stoppered, were all dead. In the case of the insects in the unstoppered bottles, it may be supposed that enough oxygen passed off from the water to satisfy the oxygen requirements of the insects.

When ready for examination, the insects are placed on a microscope slide with their venters upward. They are then covered with oil to make the wax on their bodies translucent. They are examined with the low power of the microscope for movements of the pygidium or the pharyngeal muscles; these muscles are located ventrally slightly anterior to the center of the body in the female insect. If the insects are alive, some movement can be seen in either or both of these regions of the body. If no movement can be discerned after a few minutes of examination, the insects are considered dead. In the writer's experience, red scales showing no movement are invariably proved to be dead by the appearance of their body fluids a few days later. The absence of movement in the pygidium and the pharyngeal muscles can therefore be employed as an accurate criterion of death. If no movement is found in the pharyngeal muscles, however, the pygidium should be watched carefully for a few minutes, for movements of the pygidium may sometimes be discerned several days after the pharyngeal muscles have ceased moving. Sometimes the movements of the pygidium are so feeble that only one having experience as to the nature of such movements, and hours of practice, can discern them.

The mortality effected by the absence of oxygen in the atmosphere is shown in figure 9. Each of the points in figure 9 was determined on a basis of 20 insects examined. The hot-copper method is obviously the more efficient for removing oxygen from air. When a burning match is inserted in a bottle of oxygen-free atmosphere prepared by method B (fig. 8), the flame is instantly extinguished. The flame is not so quickly extinguished by the atmosphere prepared by method A. Method A might be more efficient if the alkaline pyrogallol were continually stirred dur-

ing the course of the experiment in order to present a constantly new surface of the reducing agent to the air being bubbled through the liquid.

The experiment was repeated according to method B, but with leaves of the carob tree, *Ceratonia siliqua*, heavily infested with red scale, instead of infested lemon peels. Forty-eight hours after treatment, 486 treated insects were examined and none were found to be alive according to the criteria established for determination of live and dead insects in

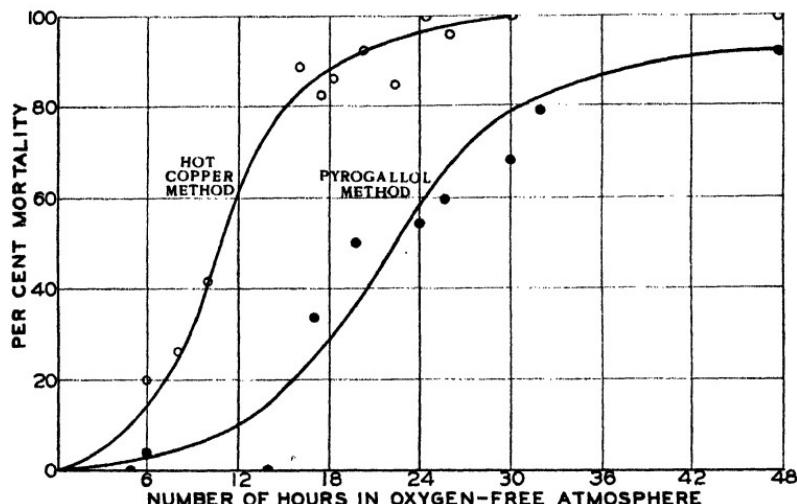


Fig. 9.—Net mortality of red scale confined in oxygen-free atmosphere.

the present series of investigations. According to the same criteria 262 insects out of 300 examined were found to be alive in the check lots; these insects had been kept in bottles from which water had been displaced by air instead of by oxygen-free atmosphere as in the treated lots.

Red scale kept in a fumigator in which a vacuum ranging from  $26\frac{1}{2}$  to  $27\frac{1}{2}$  mm of mercury was maintained for 9 hours were alive at the end of this period. A more complete vacuum could not be maintained with the equipment employed in this test.

Red scales on an infested lemon were placed in a quart jar of water for 10 days; 17 out of 20 adult scales examined with a microscope showed perceptible movements of the pygidium at the end of this period, probably having obtained sufficient oxygen from the water in which they were submerged.

Lemons infested with red scale were immersed in a grade-5 oil, then removed from the oil, and determinations made periodically of the percentage of live insects. The last live insects were found 72 hours after

the infested fruit had been immersed in oil. Carob leaves infested with red scales were immersed in grade-5 oil and a certain number of the scales were removed and examined periodically as before. Several live insects were found 60 hours after immersion, but none 72 hours after immersion. Over a thousand insects were examined in each of the above experiments.

In view of the fact that oil is practically devoid of oxygen, the greater period of survival of red scale in oil than in oxygen-free atmosphere may seem at first thought anomalous. However, deOng, Knight, and Chamberlin (1927) found that the same situation obtained with regard to the mealybug *Phenacoccus colmani* Ehr. These investigators found that the average period of lethal immersion in oil for mealybugs was nearly 7 days, while the average period of lethal immersion in hydrogen was 64 hours, or approximately 2½ days. It may be that when insects are immersed in oil, the air in their bodies and that adhering to the surface of their bodies is kept from leaving and becomes a source of oxygen for the insects for a certain period. This would be especially true of the mealybug with its waxy filaments on the body surface. If insects are immersed in an oxygen-free atmosphere, however, the air in and about their bodies becomes dissipated into the atmosphere and is then probably too dilute for their use.

*Penetration of Oil through Armor.*—Oil readily penetrates the armor of a scale insect. This may be demonstrated by removing a live adult insect, carefully scraping the body away from the armor, turning the armor with concave side upward, and placing a small amount of oil on the concavity of the armor by means of a camel's-hair brush. Sometimes the oil will penetrate through the armor in a few minutes and, with the aid of a binocular and proper lighting, may be discerned on the dorsal side of the insect. If the scales are treated as described above and placed on a microscope slide and left overnight, the oil placed in the concavities of the armors will be found in pools beneath the armors the next morning.

In order to determine whether the penetration of oil through the armor of the red scale, unaccompanied by tracheal penetration, will result fatally to the insect, small amounts of a grade-5 oil were placed on the apexes of the armors of a large number of insects by means of a camel's-hair brush. A sufficient amount of oil was usually present to spread over the dorsal surface of the armor, but not enough to penetrate under the body of the scale and enter its spiracles. This could be determined by removing the insect and noting whether or not the wax was oil-soaked under its body (fig. 10).

The treated scales were infesting mature lemons. In the present experiment a half of each lemon was marked off with ink and the scales on

this half were treated with oil, while those on the other half were left untreated as checks. The following day the experiment was repeated. Altogether 5,817 insects on 54 lemons were treated, a similar number of insects being left untreated as checks.

Four days after the scales were treated in the preceding manner, some were placed under the low power of a compound microscope and



Fig. 10.—Adult red scales broken loose from their places of attachment and turned to the right. Note difference in appearance of place of attachment and the ventral surface of the insect between scale sprayed with oil (above) and untreated scale (below). ( $\times 18$ ).

those showing signs of life were examined for the symptoms of a sub-lethal dosage of oil from the usual oil spray. The grouping of dead embryos under the armors of the adults at the bases of the pygidia was frequently observed. Thirteen days after treatment a large percentage of the live insects were giving birth to dead embryos, some of them apparently without appendages.

Of 25 treated insects examined 15 days after treatment, 8 had no crawlers under them and the remaining 17 had a total of 20 live and 87 dead crawlers, the number per insect varying from 1 to 5 live and 2 to 12 dead crawlers. Of the 25 untreated insects, 15 had no crawlers and 10

had a total of 15 live crawlers; the number per insect never exceeded 2. No dead crawlers were found under the untreated scales.

The large number of live crawlers grouped beneath the armors of treated scales indicates that the waxy margin of the scale may in some cases have been softened by the oil and may have sealed the crawlers beneath the armor, from which they could not escape.

Fifteen and 44 days after treatment the scales were examined to determine the percentage that survived the treatment of oil applied to their armors. Including the insects in the checks, a total of 4,344 were examined in this experiment. Of those examined 15 days after treatment,  $5.6 \pm 0.73$  per cent were already dead; and of those examined 44 days after treatment  $17.7 \pm 3.31$  per cent were dead. The data show that probably only a small percentage, if any, of adult red scales are killed by penetration of oil through their armors. The insects receiving enough oil on the armor to result in death are usually killed by tracheal penetration, for if more oil is placed on the armor of the scale than in the present experiment, the oil will creep off the armor and under the body of the insect and enter the spiracles. It is possible, however, that some of the insects—those that linger for a month or more after treatment before dying—may have no oil in their tracheae, but die as the result of oil penetration through the armor. If the margins of the scales are sealed with balsam so that a large amount of oil may be placed on the insects' armors without creeping under their bodies, the oil will always result in the death of the insects (see table 8).

*Effect of Sublethal Dosages of Oil on Reproduction.*—Insects that receive oil in insufficient amounts for immediate death will continue to give birth to young until they die, whether the death is attributable to the oil or to natural causes. Since the final effect of an oil spray treatment on a red-scale population must depend to some extent on its effect on the reproductive capacity of the insects not killed outright by the spray treatment, studies were made of the difference in the production of crawlers by live, untreated insects and live insects treated with oil spray. Whether the insects were alive or dead was determined by the presence or absence of movement in the pygidium or the muscles governing the pharyngeal sucking and salivary apparatus.

Some lemons infested with red scale were sprayed with grade-1 (light) oil at 2 per cent, November 16, 1933. Treated and untreated insects were examined November 29, 1933. Of 50 treated insects examined, 27 had no crawlers under them and the remaining 23 had a total of 8 live and 101 dead crawlers, the number per insect varying from 1 to 17. Of 50 untreated insects, 36 had no crawlers, 13 had a total of 19 live crawlers, and 1 had a single dead crawler.

Normally only 1 or 2 crawlers, if any, are grouped at any one time around the pygidium under the armor of the adult female. These make a rapid exit under the waxy margin of the parent and leave room for the emergence of other crawlers. However, in the case of live insects which have received a sublethal dosage of spray, a large percentage of the young are born dead (fig. 11). Many of the dead embryos found clus-

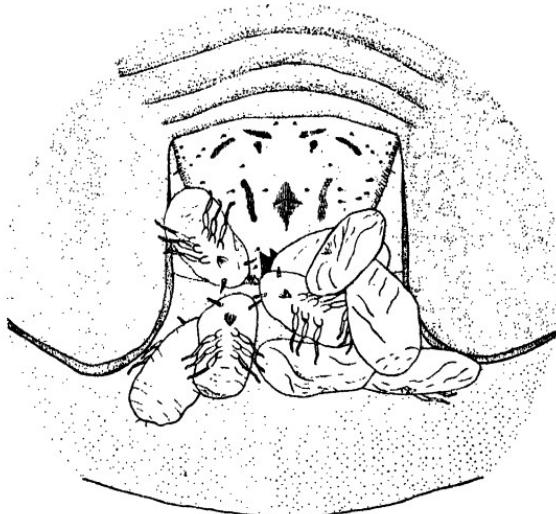


Fig. 11.—Ventral aspect of the posterior portion of a red scale which has received a sublethal dosage of oil spray. Note group of dead crawlers in the region of the pygidium.

tered about the pygidium in these cases are premature, sometimes having developed not even slight vestiges of the usual appendages. Occasionally a live crawler may be seen among the dead embryos, its exit possibly being closed by the mass of dead individuals it encounters in its path upon emergence. The writer has found as many as eighteen live crawlers under the armors of normal live insects, the posterior margins of which had been sealed with balsam. Among the insects with their entire margins sealed with balsam, which were examined 16 days after treatment (see table 8), there were an average of 4.2 live and 0.6 dead crawlers under the armor of each live adult insect.

The preponderance of dead over live crawlers in the case of the progeny of the live, treated insects does not indicate the true ratio; for the live insects probably for the most part crawl away from the body of the adult scale in the usual manner. The dead crawlers, of course, remain where born, and accumulate in considerable numbers. However, the fact

that only 8 live crawlers were found, as compared with 19 in the case of the untreated insects, indicates that a sublethal dosage of oil spray may have some value in reducing a red-scale population.

*Scales on Rough Bark.*—It is on the rough bark that the greatest percentage of insects succumb to oil sprays without actual tracheal penetration of oil. In one instance 1,000 adult scales were removed from the branches of lemon trees 24 hours after the trees had been sprayed with grade-5 oil at 1½ per cent concentration, and determinations were made as to whether or not the oil had penetrated under the bodies of the insects. The insects examined showed by their appearance that they had been alive before the spray was applied. As stated before, the presence or absence of oil can be determined by the appearance of the wax on the insects' bodies or the wax left on the tree when the insects are removed. It was found that 52.4 per cent of the insects had oil beneath their bodies and, presumably, in their tracheae. Counts made six weeks later showed a net mortality from the spray of 86.3 per cent: this indicates that 33.9 per cent of the insects were killed without tracheal penetration.

The ease with which insects are killed on the bark without tracheal penetration is to be expected in view of the probability that on the bark they are less vigorous than on other parts of the tree, as shown by their greater susceptibility to hydrocyanic acid gas and their smaller size and less vigorous appearance. On the bark, however, the least oil is available for penetration under the insect body, for it is rapidly absorbed by the dead outer layer of the bark. Consequently, despite the lower vitality of the scales living on the bark, a greater percentage survive oil-spray treatment on that part of the tree than on the green twigs, leaves, and fruit, where penetration of oil into the substratum upon which the insects live is less rapid.

Actual counts of 21,965 insects showed a survival of 17.35 per cent of the red scale on the bark, 1.36 per cent on the green twigs, 0.67 per cent on the leaves, and 7.03 per cent on the fruit of lemons sprayed with 2 per cent grade-5 oil (Ebeling, 1931). In other experiments involving the counting of 404,772 red scale, 18.20 per cent of the insects survived grade-5 oil sprays at 1½ per cent concentration on the branches and 5.51 per cent survived on the fruit. Similar ratios existed with oil sprays at other concentrations (Ebeling, 1932).

The fact that oil sprays are not able to control red scale in case of heavy infestations appears to be largely due to the rapid absorption of the oil by the rough bark, and perhaps also to the effect of the nature of the bark surface on oil deposition. On other parts of the tree the control effected by the oil sprays is satisfactory at concentrations of spray safe for use on citrus trees.

### POSSIBLE METHODS OF CONTROL

If we may judge by analogy with other insects, an understanding of the relative susceptibility of the various stages of the red scale to oil spray should form a basis for practical control measures directed against this insect. Although the several generations of red scale occurring throughout the year overlap considerably in their various stages, yet a single spray of low concentration directed against the immature stages is sufficient to even up the life stages to a large extent. It may be possible to deal with the subsequent population of scale in accordance with the principles indicated in the present paper. Experiments are being made with this possibility in view.

The addition of substances to the oil spray which would retard the penetration of oil into the bark might increase its effectiveness against the scale without increasing injury to the tree. Experiments are being made with substances which, when added to the oil spray, are expected to act as physical barriers to the penetration of oil by clogging the pores of the bark. The ideal substance for use in this connection would be one which would not be absorbed by the bark and yet could be made into a quick-breaking emulsion of high depositing qualities. Paraffin wax has been found to meet these requirements satisfactorily. Laboratory and field experiments made to date have yielded promising results and warrant a thorough study of the possibilities of this type of treatment.

The wax has been emulsified with triethanolamine stearate or triethanolamine oleate and added to the oil spray separately or combined with oil in a single emulsion. In either case small amounts of emulsified paraffin have not only greatly increased the amount of oil deposited by an oil spray, and resulted in a more uniform distribution of the oil over the leaf surface, but have also increased the insecticidal efficiency of any given amount of oil deposited.

### SUMMARY

The frequent instances of delayed lethal effect of oil have been ascribed by Smith (1932) to a "prolonged impairment of physiological processes such as might be induced by the presence of oil in the scale covering or in contact with the derm of the insect's body."

Preliminary observation had indicated that the ultimate effect of oil spray on a red-scale population is also influenced by the inhibition of the settling of crawlers for some time after the oil spray is applied. Experimental data presented in the present paper confirm this observation. They indicate that where the normal amount of oil is deposited in the

application of oil spray under orchard conditions, there is a great reduction, for at least a month after the spray is applied, in the percentage of crawlers able to settle on the foliage. Among the crawlers able to settle and form whitecaps during this interim, there is a greater mortality than where oil spray has not been applied.

Attempts at control of the red scale with dusts directed against the crawlers have been unsuccessful because of practical considerations.

All immature stages of the red scale are more easily killed by oil spray than the mature adult, probably because they are not as thoroughly sealed to the substratum upon which they rest and because their spiracles are not as far from their margins; they are thus more accessible to the oil.

The majority of adult scales are killed by tracheal penetration of the oil, although, at least on the bark, a large percentage of them die from an oil-spray treatment without tracheal penetration.

Adult red scales with their margins loosened or lifted from their substratum are more vulnerable to oil sprays than are those not treated in this manner.

It has been demonstrated that oil penetrates through the armor of the red scale.

The armors of adult scales were painted with oil, care being taken that no oil should enter beneath the bodies of the insects. In two experiments, 5.64 per cent and 17.78 per cent of the insects treated in this manner were killed by the treatment in 16 days and 44 days respectively. Those alive at the end of two weeks gave birth to a certain percentage of dead embryos and dead crawlers, as is typical of insects surviving the usual spray treatment.

Red scale were found to live in an oxygen-free atmosphere for as long as 26 hours. Immersed in oil, however, they were found to live as long as 72 hours.

A certain percentage of the progeny of adult scales receiving a sub-lethal dosage of oil spray die before they are born; some of these are born before they have developed the usual insectan appendages.

Because of the absorption of oil by the rough bark, and possibly also because of a lighter deposit of oil caused by the nature of the surface of the bark on the larger branches of citrus trees, it is on these parts of the trees that the greatest difficulty is experienced in the control of scale insects.

## ACKNOWLEDGMENTS

The writer wishes especially to express his sincere gratitude to Dr. R. H. Smith for aid and encouragement in the experimental work dealt with in the present paper and for a critical review of the manuscript. Many observations and experiments cited in this paper have been independently made by Dr. Smith, and he has also aided the writer materially by checking results and correcting certain misconceptions. Professors H. J. Quayle and W. M. Hoskins have reviewed the manuscript and offered valuable suggestions. Dr. H. B. Frost has aided in the proper interpretation of certain data, and Dr. H. D. Chapman suggested the laboratory set-up for the oxygen-deprivation experiments.

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## RECLAMATION OF BLACK-ALKALI SOILS WITH VARIOUS KINDS OF SULFUR<sup>1,2</sup>

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### INTRODUCTION

IN HARMONY with the suggestion of Lipman,<sup>(5)</sup> O'Gara<sup>(7)</sup> reported in 1917 that the application of elemental sulfur reduced the carbonate content of black-alkali soils. The results of laboratory and pot-culture tests by Hibbard<sup>(1)</sup> indicated that sulfur would be effective in the reclamation of such soils. Rudolfs,<sup>(6)</sup> and Waksman *et al.*<sup>(10)</sup> found that certain microorganisms produce active oxidation of sulfur in black-alkali soils. MacIntire, Gray, and Shaw<sup>(8)</sup> found that to a limited extent nonbiological oxidation of sulfur also takes place in soil. Kelley and Thomas<sup>(9)</sup> showed that elemental sulfur underwent oxidation in several black-alkali soils. The soils used contained soluble normal carbonate ( $\text{CO}_3$ ) varying from 0.7 to 4.0 milliequivalents per 100 grams and chloride (Cl) varying from 0.3 to 12.6 milliequivalents per 100 grams. Kelley and Thomas<sup>(9)</sup> also obtained satisfactory practical results by applying elemental sulfur to the black-alkali soil near Fresno, California. Samuels<sup>(10)</sup> reported that the application of sulfur inoculated with a certain oxidizing bacterium (*Thiobacillus thiooxidans*) gave good results in alkali-reclamation experiments at Fresno.

### KINDS OF SULFUR USED

Five different kinds of sulfur were used in the laboratory experiments and four in the field experiments here reported. These were: (1) sulfur inoculated with the oxidizing bacterium, *Thiobacillus thiooxidans*; (2) uninoculated, finely ground elemental sulfur; (3) uninoculated coarse sulfur; (4) uninoculated colloidal sulfur; (5) uninoculated sulfur concentrate.

The inoculated material contained 95 per cent pure sulfur, 80 per cent

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of which passed a 200-mesh screen. The elemental sulfur was 100 per cent sulfur, 80.07 per cent of which passed a 200-mesh screen. The particle size of the coarse sulfur ranged from 16 to 70 mesh; it was practically 100 per cent pure. The particle size of the colloidal sulfur did not exceed 25 microns. This material contained 94 per cent sulfur. The sulfur concentrate was finely ground and contained 85 per cent sulfur and about 15 per cent silica.

TABLE 1

WATER-SOLUBLE CONSTITUENTS PRESENT IN THE SOILS USED IN  
LABORATORY SULFOPICIFICATION EXPERIMENTS  
(In milliequivalents per 100 grams)

Constituent	Soil 7923	Soil 7924	Constituent	Soil 7923	Soil 7924
CO <sub>2</sub> .....	1.60	2.60	Ca.....	0.12	0.08
HCO <sub>3</sub> .....	1.30	1.25	Mg.....	0.16	0.16
Cl.....	1.97	2.40	Na.....	18.66	10.89
NO <sub>3</sub> .....	0.67	1.19	K.....	0.20	0.10
SO <sub>4</sub> .....	10.89	2.09			

TABLE 2

OXIDATION OF SULFUR AFTER TREATMENT AND INCUBATION

Amount and kinds of sulfur added, p.p.m.	Per cent oxidized after incubation for:				
	5 weeks	8 weeks	12 weeks	20 weeks	38 weeks
Soil 7923					
750 {	Inoculated sulfur.....	50.5	61.0	69.2	69.8
	Elemental sulfur.....	27.9	38.0	60.8	68.3
	Coarse sulfur.....	3.4	13.5	33.2	40.8
	Colloidal sulfur.....	34.5	43.6	65.5	68.0
	Sulfur concentrate.....	38.1	42.9	60.0	68.2
1,500 {	Inoculated sulfur.....	45.6	51.3	56.0	50.4
	Elemental sulfur.....	32.4	39.6	47.5	53.3
	Coarse sulfur.....	8.3	21.8	27.2	28.4
	Colloidal sulfur.....	37.2	42.8	48.7	52.7
	Sulfur concentrate.....	36.4	45.5	50.3	50.9
Soil 7924					
1,000 {	Inoculated sulfur.....	73.4	79.2	80.1	82.0
	Elemental sulfur.....	53.4	63.4	69.9	73.4
	Coarse sulfur.....	22.4	33.3	41.5	48.2
	Colloidal sulfur.....	58.4	65.1	68.9	70.8
	Sulfur concentrate.....	64.0	69.9	73.2	70.5
2,000 {	Inoculated sulfur.....	50.6	54.8	56.9	59.5
	Elemental sulfur.....	44.4	48.2	61.5	64.7
	Coarse sulfur.....	22.7	28.1	33.2	38.4
	Colloidal sulfur.....	39.7	52.7	61.4	66.1
	Sulfur concentrate.....	46.0	52.9	63.9	68.8

### LABORATORY EXPERIMENTS

Soil No. 7923, a Hanford fine sandy loam, and soil No. 7924, a Fresno fine sandy loam, were used in the laboratory experiments. From the analyses given in table 1, it will be seen that both soils contained appreciable amounts of soluble salts.

To soil No. 7923, which contained 1.60 milliequivalents of soluble  $\text{CO}_3$  per 100 grams, the sulfur was added at the rates of 750 and 1,500 parts

TABLE 3  
CONTENT OF NORMAL CARBONATE AFTER TREATMENT AND INCUBATION

Amount and kinds of sulfur added, p.p.m.	CO <sub>3</sub> in milliequivalents per 100 grams found after incubation for:					
	5 weeks	8 weeks	12 weeks	20 weeks	38 weeks	
Soil 7923						
750	Inoculated sulfur.....	0.60	0.45	0.30	0.27	0.22
	Elemental sulfur.....	0.90	0.60	0.55	0.35	0.25
	Coarse sulfur.....	1.50	1.45	0.85	0.70	0.55
	Colloidal sulfur.....	0.85	0.55	0.45	0.42	0.40
	Sulfur concentrate.....	0.80	0.57	0.50	0.45	0.40
1,500	Inoculated sulfur.....	0.25	0.20	0.15	0.12	0.07
	Elemental sulfur.....	0.30	0.20	0.17	0.15	0.07
	Coarse sulfur.....	0.90	0.90	0.55	0.40	0.20
	Colloidal sulfur.....	0.35	0.27	0.20	0.15	0.10
	Sulfur concentrate.....	0.32	0.20	0.20	0.17	0.07
Soil 7924						
1,000	Inoculated sulfur.....	0.20	0.17	0.15	0.12	0.10
	Elemental sulfur.....	0.60	0.45	0.15	0.10	0.05
	Coarse sulfur.....	1.60	1.37	0.95	0.85	0.35
	Colloidal sulfur.....	0.57	0.30	0.27	0.22	0.20
	Sulfur concentrate.....	0.42	0.32	0.20	0.15	0.03
2,000	Inoculated sulfur.....	0.05	0.00	0.00	0.00	0.00
	Elemental sulfur.....	0.15	0.05	0.00	0.00	0.00
	Coarse sulfur.....	0.52	0.47	0.30	0.22	0.10
	Colloidal sulfur.....	0.22	0.00	0.00	0.00	0.00
	Sulfur concentrate.....	0.10	Trace	0.00	0.00	0.00

per million (p.p.m.); and to soil No. 7924, which contained 2.60 milliequivalents of soluble  $\text{CO}_3$  per 100 grams, at rates of 1,000 and 2,000 p.p.m. The sulfur-containing materials were thoroughly mixed with 3 kilograms of the dry soil, after which distilled water was added in amounts sufficient to bring the soil to optimum water content. The soils were then incubated at room temperature in large-mouth glass bottles and samples were withdrawn from time to time for analysis.

*Amount of Sulfur Oxidized.*—Table 2 shows the amount of sulfur that had been oxidized at the various dates of sampling. During the first few weeks of the experiment the inoculated sulfur was oxidized more rapidly than the uninoculated forms. Later in the experiment the rates of oxidation became reversed, except in the case of the coarse sulfur.

TABLE 4  
BICARBONATE CONTENT AFTER TREATMENT AND INCUBATION

Amount and kinds of sulfur added, p.p.m.	HCO <sub>3</sub> in milliequivalents per 100 grams found after incubation for:					
	5 weeks	8 weeks	12 weeks	20 weeks	38 weeks	
750	Inoculated sulfur.....	0.49	0.51	0.54	0.50	0.46
	Elemental sulfur.....	0.76	0.69	0.49	0.41	0.32
	Coarse sulfur.....	1.50	0.89	0.88	0.79	0.66
	Colloidal sulfur.....	0.60	0.56	0.55	0.56	0.56
	Sulfur concentrate.....	1.21	0.65	0.60	0.57	0.48
1,500	Inoculated sulfur.....	0.41	0.35	0.50	0.50	0.50
	Elemental sulfur.....	0.44	0.52	0.48	0.41	0.31
	Coarse sulfur.....	0.70	0.68	0.69	0.62	0.50
	Colloidal sulfur.....	0.61	0.51	0.50	0.50	0.51
	Sulfur concentrate.....	0.59	0.56	0.54	0.50	0.51
1,000	Inoculated sulfur.....	0.51	0.57	0.32	0.34	0.39
	Elemental sulfur.....	0.70	0.56	0.49	0.46	0.45
	Coarse sulfur.....	0.95	0.60	0.62	0.59	0.54
	Colloidal sulfur.....	0.57	0.57	0.40	0.40	0.40
	Sulfur concentrate.....	0.59	0.59	0.32	0.32	0.34
2,000	Inoculated sulfur.....	0.36	0.34	0.30	0.29	0.25
	Elemental sulfur.....	0.42	0.45	0.29	0.26	0.22
	Coarse sulfur.....	1.12	0.70	0.49	0.45	0.31
	Colloidal sulfur.....	0.88	0.38	0.29	0.29	0.27
	Sulfur concentrate.....	0.39	0.38	0.27	0.29	0.27

With both soils the coarse sulfur underwent oxidation more slowly than any of the fine-grained materials, showing that small particles become oxidized more rapidly than large ones. This was to be expected because the rate of oxidation is a surface phenomenon and depends upon the amount of exposed surface.

It is interesting to note that with each soil the rate of oxidation was roughly proportional to the amount of sulfur added.

*Effect of Sulfur on Soluble CO<sub>3</sub> and HCO<sub>3</sub>.*—Table 3 gives the amounts of soluble normal carbonate (CO<sub>3</sub>) found at the various dates of sampling. The content of CO<sub>3</sub> was reduced in every case, but at markedly

different rates. The decrease in soluble  $\text{CO}_3$  was roughly proportional to the amount of sulfur that was oxidized. Since, as shown in table 2, the inoculated sulfur was oxidized more rapidly than the other forms during the first few weeks of the experiment, it is interesting to find that the soluble  $\text{CO}_3$  decreased proportionately. Towards the close of

TABLE 5  
WATER-SOLUBLE CALCIUM CONTENT AFTER TREATMENT AND INCUBATION

Amount and kinds of sulfur added, p.p.m.	Water-soluble Ca in milliequivalents per 100 grams found after incubation for:				
	5 weeks	8 weeks	12 weeks	38 weeks	
Soil 7923					
750	Inoculated sulfur.....	Trace	0.22	0.23	0.31
	Elemental sulfur.....	Trace	0.27	0.33	
	Coarse sulfur.....	Trace	Trace	Trace	0.23
	Colloidal sulfur.....	Trace	Trace	0.10	0.27
	Sulfur concentrate.....	Trace	Trace	0.05	0.32
1,500	Inoculated sulfur.....	0.15	0.40	0.51	2.36
	Elemental sulfur.....	0.12	0.37	0.47	1.01
	Coarse sulfur.....	Trace	Trace	0.22	0.27
	Colloidal sulfur.....	0.10	0.10	0.29	1.05
	Sulfur concentrate.....	Trace	Trace	0.35	1.62
Soil 7924					
1,000	Inoculated sulfur.....	0.12	0.15	0.10	0.21
	Elemental sulfur.....	Trace	Trace	0.05	0.14
	Coarse sulfur.....	Trace	Trace	Trace	0.02
	Colloidal sulfur.....	Trace	0.10	0.15	0.20
	Sulfur concentrate.....	Trace	0.09	0.20	0.27
2,000	Inoculated sulfur.....	0.58	1.02	2.37	3.45
	Elemental sulfur.....	0.35	0.39	1.07	2.49
	Coarse sulfur.....	Trace	0.13	0.06	0.37
	Colloidal sulfur.....	0.23	0.61	1.40	2.46
	Sulfur concentrate.....	0.40	0.70	1.35	3.24

the experiment, the finely ground elemental sulfur, the colloidal form, and the sulfur concentrate, none of which were inoculated, produced fully as great reduction in the soluble  $\text{CO}_3$  as the inoculated sulfur. On the other hand, the coarse sulfur was less effective at all stages of the experiment. As shown in table 4, the soluble bicarbonate ( $\text{HCO}_3$ ) was affected like the soluble normal carbonate ( $\text{CO}_3$ ). (See table 3).

*Effect of Sulfur on the Water-Soluble Calcium.*—Table 5 gives the content of water-soluble calcium in the various samples. A comparison of the analyses in this table with those in table 3 shows that not more

than a small amount of water-soluble calcium was found until practically all the  $\text{CO}_3$  had been neutralized. After the  $\text{CO}_3$  had been neutralized, however, appreciable amounts of water-soluble calcium were found in all of the samples.

### FIELD EXPERIMENTS

The inoculated sulfur and three of the forms of uninoculated sulfur, namely, elemental, coarse, and colloidal sulfur, were used in replicated plot experiments. The sulfur materials were added at the rate of 1 ton of actual sulfur per acre to plots approximately  $\frac{1}{4}$  acre in size. Two plots were left untreated as checks.



Fig. 1.—Condition of the field plots before the sulfur experiments were begun (photographed March, 1927). Note failure of the barley crop sown the previous fall.

The sulfur materials were applied in July, 1927, and were plowed under to a depth of 3 or 4 inches. The soil was irrigated and cultivated during the remainder of the summer and the fall of that year (fig. 1).

The flooding system of irrigation was used, the entire surface of each plot being covered with water at each application. This produced more or less leaching of the soil.

*Soluble  $\text{CO}_3$  and  $\text{HCO}_3$  in Field-Plot Soils.*—Table 6 gives the content of soluble  $\text{CO}_3$  in samples of soil taken to a depth of one, two, three, and four feet respectively. One set of soil samples was taken before the sulfur treatments were applied, a second set 6 months later, and a third set 18 months after the sulfur was applied.

Within 6 months after the applications had been made, practically all of the soluble  $\text{CO}_3$  had been removed from the surface foot of soil in each of the sulfur-treated plots. The irrigation water had also leached a

part of the  $\text{CO}_3$  from the surface foot of the untreated plot. The sulfur treatments caused considerable reduction in soluble  $\text{CO}_3$  in the second foot also, but the effects were only slight in the third and fourth feet.

Table 7 shows that the soluble  $\text{HCO}_3$  was only slightly affected by any of the sulfur materials.

*Soluble  $\text{SO}_4$  in Field-Plot Soils.*—Table 8 gives the content of soluble

TABLE 6

CONTENT OF NORMAL CARBONATE ( $\text{CO}_3$ ) IN FIELD-PILOT SOILS TREATED WITH  
DIFFERENT KINDS OF SULFUR  
(In milliequivalents per 100 grams)

Time of analysis	0-12 inch depth	12-24 inch depth	24-36 inch depth	36-48 inch depth
Inoculated sulfur				
Before treatment.....	0.62	0.50	0.42	0.30
6 months after treatment.....	0.00	0.21	0.60	0.50
18 months after treatment.....	0.00	0.17	0.50	0.82
Elemental sulfur				
Before treatment.....	0.92	0.87	0.35	0.52
6 months after treatment.....	0.00	0.52	0.87	0.57
18 months after treatment.....	0.00	0.42	0.80	0.88
Coarse sulfur				
Before treatment.....	0.80	0.90	0.95	0.80
6 months after treatment.....	0.05	0.67	0.95	0.60
18 months after treatment.....	0.00	0.57	0.87	0.47
Colloidal sulfur				
Before treatment.....	0.67	1.27	1.25	0.85
6 months after treatment.....	0.05	0.75	1.12	0.80
18 months after treatment.....	0.00	0.57	1.22	1.02
Untreated				
Before time of treating the other plots.....	0.97	1.07	1.25	0.92
6 months later.....	0.75	0.92	1.12	0.87
18 months later.....	0.47	0.95	1.37	1.22

sulfate ( $\text{SO}_4$ ) in the field-plot soils. Within 6 months after the sulfur applications, the  $\text{SO}_4$  content of all of the treated plots had increased. Since these soils were irrigated by flooding, they must have been leached to some extent. This is indicated by the increase in the  $\text{SO}_4$  content in the subsoil. In the untreated plots, on the other hand, the  $\text{SO}_4$  content

was reduced throughout the profile, an indication that more or less leaching took place.

*Soluble Cl in Field-Plot Soils.*—Table 9 gives the content of soluble chloride (Cl) in the field-plot soils. The chloride content was reduced substantially throughout the entire profile of each plot. This was fully as pronounced in the untreated as in the sulfur-treated plots.

TABLE 7

BICARBONATE ( $\text{HCO}_3$ ) CONTENT OF FIELD- PLOT SOILS TREATED WITH  
DIFFERENT KINDS OF SULFUR  
(In milliequivalents per 100 grams)

Time of analysis	0-12 inch depth	12-24 inch depth	24-36 inch depth	36-48 inch depth
Inoculated sulfur				
Before treatment.....	0.55	0.45	0.58	0.56
6 months after treatment.....	0.35	0.51	0.54	0.49
18 months after treatment.....	0.39	0.44	0.52	0.44
Elemental sulfur				
Before treatment.....	0.56	0.65	0.45	0.44
6 months after treatment.....	0.48	0.64	0.55	0.49
18 months after treatment.....	0.42	0.45	0.46	0.36
Coarse sulfur				
Before treatment.....	0.60	0.50	0.62	0.50
6 months after treatment.....	0.45	0.62	0.83	0.46
18 months after treatment.....	0.37	0.51	0.55	0.49
Colloidal sulfur				
Before treatment.....	0.50	0.57	0.46	0.58
6 months after treatment.....	0.44	0.55	0.64	0.53
18 months after treatment.....	0.44	0.56	0.53	0.55
Untreated				
Before time of treating the other plots.....	0.41	0.48	0.52	0.52
6 months later.....	0.52	0.56	0.67	0.57
18 months later.....	0.46	0.40	0.49	0.45

TABLE 8

SULFATE ( $\text{SO}_4$ ) CONTENT OF FIELD-PILOT SOILS TREATED WITH  
DIFFERENT KINDS OF SULFUR  
(In milliequivalents per 100 grams)

Time of analysis	0-12 inch depth	12-24 inch depth	24-36 inch depth	36-48 inch depth
Inoculated sulfur				
Before treatment.....	0.13	0.40	1.00	0.84
6 months after treatment.....	1.67	0.83	0.73	1.04
18 months after treatment.....	0.16	0.44	0.59	0.86
Elemental sulfur				
Before treatment.....	0.16	0.38	0.38	0.45
6 months after treatment.....	1.26	0.51	0.68	0.78
18 months after treatment.....	0.09	0.11	0.24	0.28
Coarse sulfur				
Before treatment.....	0.13	0.22	0.16	0.19
6 months after treatment.....	0.99	0.59	0.49	0.39
18 months after treatment.....	0.13	0.21	0.36	0.39
Colloidal sulfur				
Before treatment.....	0.12	0.31	0.44	0.22
6 months after treatment.....	1.21	0.71	0.65	0.60
18 months after treatment.....	0.29	0.32	0.48	0.41
Untreated				
Before time of treating the other plots.....	0.15	0.13	0.26	0.30
6 months later.....	0.06	0.07	0.11	0.24
18 months later.....	0.04	0.04	0.07	0.06

TABLE 9

CHLORINE (Cl) CONTENT OF FIELD-PILOT SOILS TREATED WITH  
 DIFFERENT KINDS OF SULFUR  
 (In milliequivalents per 100 grams)

Time of analysis	0-12 inch depth	12-24 inch depth	24-36 inch depth	36-48 inch depth
Inoculated sulfur				
Before treatment.....	0.35	0.91	1.95	2.26
6 months after treatment.....	0.21	0.25	0.49	1.55
18 months after treatment.....	0.19	0.19	0.26	0.60
Elemental sulfur				
Before treatment.....	0.45	1.02	0.55	1.00
6 months after treatment.....	0.21	0.21	0.45	0.75
18 months after treatment.....	0.17	0.20	0.25	0.22
Coarse sulfur				
Before treatment.....	0.37	0.60	0.56	0.65
6 months after treatment.....	0.16	0.19	0.39	0.55
18 months after treatment.....	0.15	0.14	0.17	0.15
Colloidal sulfur				
Before treatment.....	0.36	0.81	1.15	0.80
6 months after treatment.....	0.20	0.24	0.51	0.65
18 months after treatment.....	0.17	0.25	0.27	0.29
Untreated				
Before time of treating the other plots.....	0.49	0.39	0.85	0.87
6 months later.....	0.12	0.12	0.25	0.26
18 months later.....	0.10	0.14	0.19	0.21

## GROWTH OF CROPS ON FIELD PLOTS

Hubam clover was grown the first season after the sulfur materials were applied. The crop made good growth on all of the sulfur-treated plots. On the untreated plots the seed did not germinate in certain areas, and the soil remained bare, not even supporting the most alkali-resistant plants. The Hubam clover was plowed under as a green manure in September, 1928.

Alfalfa was sown on the plots the following spring. The seed germinated well, a complete stand being obtained on all of the treated plots

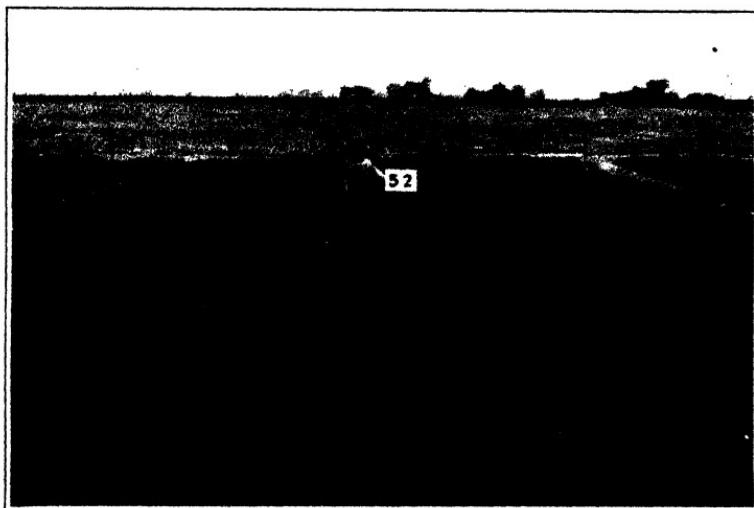


Fig. 2.—Alfalfa on the plot treated with coarse sulfur. The alfalfa was sown February 21, 1929, and photographed May 15, 1929.

(figs. 2, 3, 4, and 5). On the untreated plots the stand was uneven (fig. 6). Many of the young alfalfa plants died on the areas that had failed to support a good growth of Hubam clover the previous season.

As shown in the figures, good yields of alfalfa hay have been obtained on all of the sulfur-treated plots, no significant difference being found between the different kinds of sulfur. Every one of the sulfurs produced satisfactory results (table 10).

In 1933 oats were grown and an excellent yield of hay was produced on all the sulfur-treated plots. A good yield of oats was secured on certain parts of the check plots also. This may be attributed to the extreme variability of alkali soil in general as discussed by Kelley.<sup>10</sup> At the

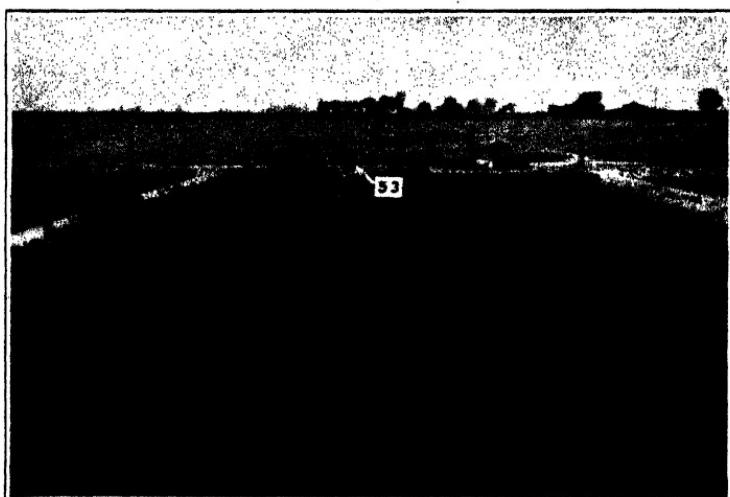


Fig. 3.—Alfalfa on plot treated with colloidal sulfur. The alfalfa was sown February 21, 1929, and photographed May 15, 1929.

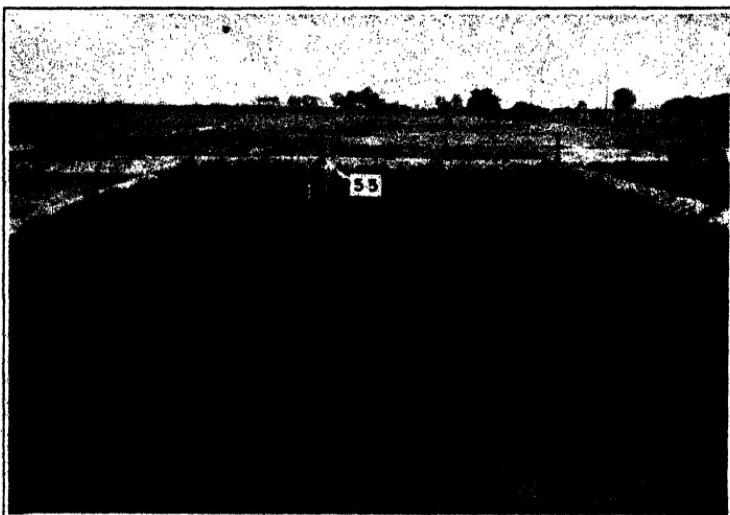


Fig. 4.—Alfalfa on the plot treated with inoculated sulfur. The alfalfa was sown February 21, 1929, and photographed May 15, 1929.

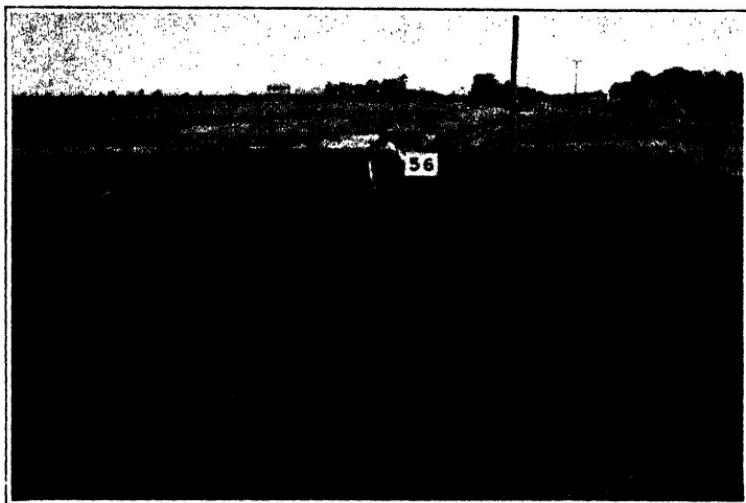


Fig. 5.—Alfalfa on the plot treated with elemental sulfur. The alfalfa was sown February 21, 1929, and photographed May 15, 1929.

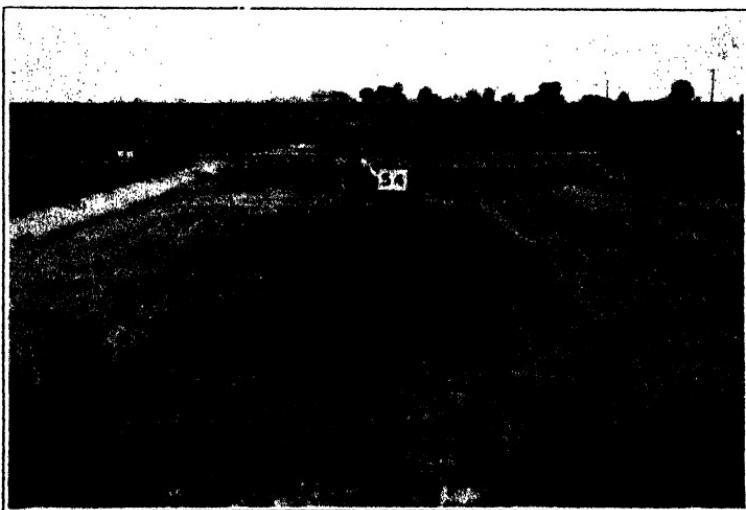


Fig. 6.—Alfalfa on the untreated plot. The alfalfa was sown February 21, 1929, and photographed May 15, 1929. Most of the plants died soon after the date the photograph was taken.

beginning of the experiment the soil in certain portions of each plot contained much less black alkali than the soil in other portions. Some of the soil in the untreated plots contained only a small amount of black alkali and other soluble salts, and was more permeable than where larger amounts of black alkali occurred. In passing through such soil, the irrigation water naturally leached out the soluble salts to a greater extent than in the remainder of the plot. On places of this kind in the check plots the alfalfa and oats made good growth after the concentration of soluble salts had been sufficiently reduced.

TABLE 10

## CROP YIELDS IN POUNDS PER ACRE AS AffECTED BY DIFFERENT KINDS OF SULFUR

Year	Inoculated sulfur	Elemental sulfur	Coarse sulfur	Colloidal sulfur	Untreated
Alfalfa hay					
1929.....	10,937	10,000	10,058	10,097	4,082
1930.....	19,334	20,252	20,995	21,443	10,605
1931.....	17,225	16,867	20,495	21,412	14,772
1932*.....	7,362	7,598	8,887	8,946	5,918
Oat hay					
933.....	5,195	5,235	5,478	5,488	4,179

\* The yields obtained in 1932 were from three cuttings. Six cuttings were made in 1930, and seven in 1931.

## SUMMARY

For several years good results have been obtained with the use of sulfur as a treatment of black-alkali soils. Both uninoculated and inoculated sulfurs have been used. All of the sulfurs used gave good results both in the laboratory and in the field, but the rates of oxidation were different. The rate of oxidation of sulfur inoculated with *Thiobacillus thiooxidans* was greater than that of the uninoculated sulfurs for the first 8 weeks of the experiment. Uninoculated sulfurs with particles similar in size to those of the inoculated sulfur underwent fully as rapid oxidation after the lapse of 8 to 10 weeks as the inoculated sulfur.

The rate of oxidation of the coarse sulfur was slower than that of any of the finer-grained sulfurs because of the difference in particle size. In the course of time, however, the coarse sulfur gave as good results as the other sulfurs. This was shown by the fact that 18 months after the coarse sulfur had been applied, the CO<sub>2</sub> content of the soil was reduced in each foot layer to a depth of 4 feet.

These results show that whatever form of sulfur is used it should be applied on the basis of actual sulfur content.

The laboratory and field experiments both indicate that various kinds of sulfur will be effective in the treatment of black-alkali soils which contain lime or other readily decomposable calcium minerals; however, the sulfur should be finely pulverized in order that the material may be evenly distributed. With the soils used in these experiments, at least, artificial inoculation of sulfur is unnecessary.

In order to secure the best results with sulfur, the soil should be leveled before application is made. When applied, the sulfur should be mixed with the soil by shallow plowing or disk ing, after which irrigation and cultivation should be as frequent as is necessary to keep the soil moist and well aerated.

Good drainage conditions are necessary in order that the soluble salts may be removed from the soil by leaching. In some cases it may be possible to leach the soluble salts from the root zone by the regular irrigations, while in other places it is necessary to subject the soil to heavy flooding in order to remove the salts.

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# H I L G A R D I A

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## DETERMINING CHANGES IN STORED PEAS BY USE OF A REFERENCE ELEMENT<sup>1</sup>

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### INTRODUCTION

THE FRESH PEA CROP is of considerable economic importance in California. As most of it is shipped to distant markets, there is danger of the product's being subject to conditions deleterious to edible quality. To a certain extent the continued success of the industry depends upon placing a high-quality commodity on the table of the consumer. In an effort to help in guiding commercial practices in this direction, studies have been made on the fundamental changes occurring in the peas and pods after harvest.

As a rule the results of analyses presented by most investigators studying changes in stored material have been expressed as percentages of dry matter. When this method of expression is used, it is difficult to secure a true picture of the absolute changes that are taking place because the variables being studied, such as sugars and starches, are compared with another variable—total dry matter. Kertesz<sup>193</sup> has recently pointed out some of the errors commonly occurring in the accumulation and presentation of data on stored material.

In these studies the authors have been interested in determining the absolute changes occurring in both pods and peas in storage and the transfer of material from one to the other. The method that seemed most

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<sup>5</sup> Superscript numbers in parentheses refer to papers in Literature Cited at the end of the paper.

accurate was to use some element that does not undergo any change in absolute amount during storage as a reference weight with which to compare those compounds that do undergo changes during storage.

The variety used in these studies was Laxton's Progress and the crop was grown at Davis in the spring of 1931.

### EXPERIMENTAL PROCEDURE

Material for the master and storage samples was selected from a 25-kg field sample harvested in the early morning of the day the storage experiment was begun. The fruit (that is, the pods containing peas) was picked at the age customary for market peas. Care was used to harvest fruit as uniform in stage of maturity as possible. Each sample consisted of approximately 500 grams of fresh fruit. Eight samples were used for checks, and the average of these checks constituted what is hereafter called the master sample. These eight samples were shelled immediately after harvest, and the pods and peas preserved separately for analysis as previously described by Bisson and Jones.<sup>10</sup> To reduce the chance for errors due to loss of moisture, translocation, and transpiration occurring during preparation of the samples for storage, the remaining material was kept cool. All the samples for storage at 3½° C were prepared first, the samples of the 25° shelled series next, and the samples of the 25° unshelled series last. Twenty samples of shelled and twenty of unshelled fruit were used for the storage studies at 3½°; fifteen of each for the 25° series. Quintuplicate samples from each of the two series were removed at intervals as indicated in the tables. The fruits of the unshelled stored samples were shelled immediately after removal from the storage rooms. The pods and peas from both series were preserved separately for analysis.

The samples of peas and pods were analyzed for dry matter and some of its constituents according to methods given in an earlier paper.<sup>10</sup> Phosphorus and magnesium were determined volumetrically, and the results examined for uniformity. Phosphorus, which proved to be the most consistently uniform in percentage composition, was then chosen as the basis for calculating the absolute weights of the various components as indicated by Bisson and Jones.<sup>10</sup> The average percentage of phosphorus in the peas of the eight check samples was 0.586 with a standard error of  $\pm 0.006$ . The average percentage of phosphorus in the pods of the eight check samples was 0.238 with a standard error of  $\pm 0.004$ .

## METHODS OF CALCULATION

In calculating the results for the initial weight of dry matter from the experimental data, the authors assumed that a constant initial ratio of average weight of phosphorus to average weight of the total dry matter and likewise the same initial distribution of phosphorus between peas

TABLE 1  
CORRECTED WEIGHT OF PHOSPHORUS, IN GRAMS, IN INITIAL SAMPLES

Part of fruit	Hours in storage	Actual weight of phosphorus in sample	Correction, $e^*$	Corrected weight of phosphorus in initial sample	Hours in storage	Actual weight of phosphorus in sample	Correction, $e^*$	Corrected weight of phosphorus in initial sample
Peas.....	0	$n_1$ 0.2924	0.0000	$m_1$ 0.2924	0	$n_1$ 0.2924	0.0000	$m_1$ 0.2924
	22	.2943	+.0004	.2939	20	.3050	+.0086	.2964
	70	.2981	+.0036	.2945	65	.3435	+.0422	.3013
	112	.3024	+.0026	.2998	111	.3635	+.0661	.2974
Pods.....	0	$n_2$ .1024	.0000	$m_2$ .1024	0	$n_2$ .1024	.0000	$m_2$ .1024
	22	.1026	-.0004	.1030	20	.0952	-.0086	.1038
	70	.0995	-.0036	.1031	65	.0633	-.0422	.1055
	112	0.1024	-0.0026	0.1050	111	0.0381	-0.0661	0.1042
Peas.....	0	$n_1$ 0.2924	0.0000	$m_1$ 0.2924	0	$n_1$ 0.2924	0.0000	$m_1$ 0.2924
	46	.2942	+.0002	.2940	46	.3057	+.0049	.3008
	95	.2870	-.0031	.2901	93	.3053	+.0079	.2974
	143	.2947	+.0008	.2941	141	.3100	+.0102	.2998
	188	.2910	-.0005	.2915	187	.3070	+.0101	.2969
Pods.....	0	$n_2$ .1024	.0000	$m_2$ .1024	0	$n_2$ .1024	.0000	$m_2$ .1024
	46	.1028	-.0002	.1030	46	.1004	-.0049	.1053
	95	.1047	+.0031	.1016	93	.0963	-.0079	.1042
	143	.1025	-.0006	.1031	141	.0948	-.0102	.1050
	188	0.1026	+0.0005	0.1021	187	0.0939	-0.0101	0.1040

\* For meaning of  $e$  see page 147.

and pods existed in all the storage and master samples, within the limit of error of the procedure—an assumption justified by the uniformity of this ratio and the distribution in the eight separate check samples. The initial weight of dry matter was calculated from the ratio of the weight of phosphorus to the weight of dry matter in the master sample, and the calculated initial average weight of phosphorus in each of the storage samples.

*Calculating Actual Average Weights of Phosphorus.*—The actual average weights,  $n_1$  and  $n_2$ , of phosphorus present in the master sample and storage samples are given in table 1, columns 2 and 6. These values were obtained by multiplying the average percentages of phosphorus as given in columns 2 and 6 of table 11 by the corresponding actual average weights of dry matter as given in column 2 of tables 2, 3, 4, and 5, and dividing the quotient by 100. In the master sample the weight of dry

TABLE 2\*  
WEIGHT OF PHOSPHORUS AND DRY MATTER, IN GRAMS, OF PEAS AND PODS  
STORED SHELLLED AT 25° C

Part of fruit	Hours in storage	Actual weight of dry matter after storage	Weight of phosphorus		Weight of dry matter			
			Actual	Corrected initial	Calculated initial	After storage of corrected initial sample	After storage of sample equal to master sample	Loss during storage of sample equal to master sample
	1	2	3	4	5	6	7	8
Peas	0	$w_1$ 49.9†	$n_1$ 0.292	$m_1$ 0.292	$b_1$ 49.9†	$r_1$ 49.9†	$s_1$ 49.9†	$l_1$ 0.0
	22	47.7	.294	.294	50.2	47.7	47.4	2.5
	70	46.3	.298	.295	50.4	45.8	45.4	4.5
	112	45.4	.302	.300	51.2	45.1	43.9	6.0
Pods	0	$w_2$ 43.0†	$n_2$ .102	$m_2$ .102	$b_2$ 43.0†	$r_2$ 43.0†	$s_2$ 43.0†	$l_2$ 0.0
	22	41.4	.103	.103	43.4	41.4	41.0	2.0
	70	38.6	.100	.103	43.4	39.8	39.4	3.6
	112	36.9	.102	.105	44.3	38.0	36.9	6.1
Peas and pods	0	92.9†	.394	.394	92.9†	92.9†	92.9†	0.0
	22	89.1	.397	.397	93.6	89.1	88.4	4.5
	70	84.9	.398	.398	93.8	85.6	84.8	8.1
	112	82.3	0.404	0.405	95.5	83.1	80.8	12.1

\* Columns 1 through 3 are experimental data; 4 through 8, calculated results.

† Actual weight of dry matter in master sample.

matter in the shelled peas was 49.9 grams, of which 0.292 gram was phosphorus (table 2, cols. 2 and 3). The weight of dry matter in the pods of the master sample was 43.0 grams, of which 0.102 gram was phosphorus.

*Calculating Initial Weights of Phosphorus.*—The ratio of the weight of phosphorus in the peas to that in the pods of the master sample served as the basis for calculating the initial distribution of the phosphorus between peas and pods, or the initial weights of phosphorus in peas and pods, for all samples from their actual weights of phosphorus. The actual weight of phosphorus cannot be used directly as the basis for calculating the initial weight of dry matter because varying amounts of phosphorus compounds were translocated from pods to peas during the

time required to prepare the shelled samples for storage. Much larger amounts were transferred from pods to peas in the unshelled series during storage.

The equation by which the weight of phosphorus translocated from pods to peas was determined for each sample is  $\frac{n_1 - e}{n_2 + e} = 2.855$ , from which

$$e = 0.2594 n_1 - 0.7405 n_2 \quad 1$$

In this equation  $n_1$  represents the actual weight of phosphorus in the peas,  $n_2$  the actual weight of phosphorus in the pods,  $e$  the weight of

TABLE 3\*  
WEIGHT OF PHOSPHORUS AND DRY MATTER, IN GRAMS, OF PEAS AND PODS  
STORED UNSHELLED AT 25° C

Part of fruit	Hours in storage	Actual weight of dry matter after storage	Weight of phosphorus		Weight of dry matter		
			Actual	Corrected initial	Calculated initial	After storage of initial sample equal to master sample	Changes during storage of sample equal to master sample
	1	2	3	4	5	6	7
Peas.....	0	$w_1$ 49.9	$n_1$ 0.292	$m_1$ 0.292	$b_1$ 49.9	$v_1$ 49.9	$d_1$ 0.0
	20	50.0	.305	.296	50.6	49.3	-0.6
	65	55.9	.344	.301	51.4	54.2	+4.3
	111	57.9	.364	.297	50.8	56.9	+7.0
Pods.....	0	$w_2$ 43.0	$n_2$ .102	$m_2$ .102	$b_2$ 43.0	$v_2$ 43.0	$d_2$ 0.0
	20	41.0	.095	.104	43.8	40.2	-2.8
	65	30.7	.063	.106	44.7	29.5	-13.5
	111	25.1	.038	.104	43.8	24.6	-18.4
Peas and pods.....	0	92.9	.394	.394	92.9	92.9	0.0
	20	91.0	.400	.400	94.4	89.5	-3.4
	65	86.6	.407	.407	98.1	83.7	-9.2
	111	83.0	0.402	0.401	94.6	81.5	-11.4

\* Columns 1 through 3 are experimental data; 4 through 7, calculated results.

phosphorus translocated during the preliminary preparation period for the shelled series or during the storage period for the unshelled series. The constant 2.855 is the ratio of the weight of phosphorus in the peas to that in the pods of the master samples, table 1, column 2. A specific application of this equation, using the data for the 65-hour 25° C unshelled stored sample, gave by substitution  $e = 0.2594 \times 0.3435 - 0.7405 \times 0.0633$  and a value for  $e$  of 0.0422 gram, the weight of the phosphorus translocated. The values of  $e$  were subtracted algebraically from the corresponding actual weights of phosphorus in the peas and

pods in columns 2 and 6 of table 1, and the corrected weights of phosphorus recorded as  $m_1$  and  $m_2$  in columns 4 and 8 of table 1. The initial weights of phosphorus are expressed to one place beyond the significant figure used in later calculations, simply to indicate that for some samples the amount translocated was not significant. The values of  $n_1$  and  $n_2$  and  $m_1$  and  $m_2$  used in all later calculations were expressed to three decimal places and are recorded in columns 3 and 4 of tables 2 to 5 inclusive.

TABLE 4\*  
WEIGHT OF PHOSPHORUS AND DRY MATTER, IN GRAMS, OF PEAS AND PODS  
STORED SHELLED AT  $3\frac{1}{2}^{\circ}$  C

Part of fruit	Hours in storage	Actual weight of dry matter after storage	Weight of phosphorus		Weight of dry matter			
			Actual	Corrected initial	Calculated initial	After storage of corrected initial sample	After storage of sample equal to master sample	Loss during storage of sample equal to master sample
	1	2	3	4	5	6	7	8
Peas	0	$w_1$ 49.9	$n_1$ 0.292	$m_1$ 0.292	$b_1$ 49.9	$r_1$ 49.9	$s_1$ 49.9	$l_1$ 0.0
	46	49.3	.294	.294	50.2	49.3	49.0	0.9
	95	47.6	.287	.290	49.5	48.1	48.4	1.5
	143	48.1	.295	.294	50.2	47.9	47.6	2.3
	188	47.4	.291	.291	49.7	47.4	47.6	2.3
Pods	0	$w_2$ 43.0	$n_2$ .102	$m_2$ .102	$b_2$ 43.0	$r_2$ 43.0	$s_2$ 43.0	$l_2$ 0.0
	46	42.2	.103	.103	43.4	42.2	41.8	1.2
	95	42.7	.105	.102	43.0	41.5	41.5	1.5
	143	42.0	.102	.103	43.4	42.4	42.0	1.0
	188	42.0	.103	.102	43.0	41.6	41.6	1.4
Peas and pods	0	92.9	.394	.394	92.9	92.9	92.9	0.0
	46	91.5	.397	.397	93.6	91.5	90.8	2.1
	95	90.3	.392	.392	92.5	89.6	89.9	3.0
	143	90.1	.397	.397	93.6	90.3	89.6	3.3
	188	89.4	0.394	0.393	92.7	89.0	89.2	3.7

\* Columns 1 through 3 are experimental data; 4 through 8, calculated results.

*Calculating Initial Weights of Dry Matter for Both Shelled and Unshelled Series.*—The initial weights of dry matter  $b_1$ , in all storage samples of peas were then calculated by a proportion; the calculated initial weight of phosphorus  $m_1$  in any sample was multiplied by a ratio which was the weight of dry matter (49.9 grams) divided by the weight of phosphorus (0.292 gram) in the peas of the master sample, as given by the equation

$$b_1 = m_1 \times \frac{49.9}{0.292} = m_1 \times 170.8 \quad 2$$

The initial weights of dry matter in the pods  $b_2$  were calculated by an equation of similar form, using the corresponding data for pods. The

ratio used was  $\frac{43.0}{0.102} = 421.5$ . Equation 2 was derived on the assumption that the ratio of the initial weight of dry matter to the calculated initial weight of phosphorus in all samples was the same as that in the master sample for both peas and pods. The calculated initial weights are not comparable with the weights of dry matter given in column 2 of tables

TABLE 5\*

WEIGHT OF PHOSPHORUS AND DRY MATTER, IN GRAMS, OF PEAS AND PODS  
STORED UNSHELLED AT  $3\frac{1}{2}$ ° C

Part of fruit	Hours in storage	Actual weight of dry matter after storage	Weight of phosphorus		Weight of dry matter		
			Actual	Corrected initial	Calculated initial	After storage of initial sample equal to master sample	Changes during storage of sample equal to master sample
	1	2	3	4	5	6	7
Peas.....	0	w <sub>1</sub> 49.9	n <sub>1</sub> 0.292	m <sub>1</sub> 0.292	b <sub>1</sub> 49.9	v <sub>1</sub> 49.9	d <sub>1</sub> 0.0
	46	51.5	.306	.301	51.4	50.0	+0.1
	93	51.3	.305	.297	50.7	50.4	+0.5
	141	52.3	.310	.300	51.2	50.9	+1.0
	187	51.7	.307	.297	50.7	50.8	+0.9
Pods.....	0	w <sub>2</sub> 48.0	n <sub>2</sub> .102	m <sub>2</sub> .102	b <sub>2</sub> 43.0	v <sub>2</sub> 43.0	d <sub>2</sub> 0.0
	46	41.8	.100	.105	44.3	40.6	-2.4
	93	40.6	.098	.104	43.8	39.8	-3.2
	141	39.3	.095	.105	44.3	38.2	-4.8
	187	38.8	.094	.104	43.8	38.0	-5.0
Peas and pods.	0	92.9	.394	.394	92.9	92.9	0.0
	46	93.3	.406	.408	95.7	90.6	-2.3
	93	91.9	.401	.401	94.5	90.2	-2.7
	141	91.6	.405	.405	95.5	89.1	-3.8
	187	90.5	0.401	0.401	94.5	88.8	-4.1

\* Columns 1 through 3 are experimental data; 4 through 7, calculated results.

2 to 5 inclusive, because these are weights of dry matter which resulted after changes had taken place during storage.

The calculations for the 112-hour sample stored shelled at 25° C (table 2) gave  $0.300 \times 170.8 = 51.2$  grams for the initial weight of dry matter in the peas and  $0.105 \times 421.5 = 44.3$  grams for the initial weight of dry matter in the pods. The initial weight of dry matter in the whole fruit was therefore 95.5 grams. The total weight of dry matter in the whole fruit of the master sample, however, was 92.9 grams. The initial weights of dry matter in the samples of all the storage series, calculated in this manner, are recorded in column 5 of tables 2, 3, 4, and 5.

*Calculation of Final Results for the Shelled Series: Weight of Dry Matter.*—The final weights of dry matter, or those resulting after storage from samples of shelled peas whose dry weights were equal to the calculated initial dry weights given in column 5, tables 2 and 4, were obtained by substituting values in the equation

$$r_1 = w_1 \times \frac{m_1}{n_1}, \quad 3$$

in which for any sample of the shelled series,  $r_1$  represents the calculated dry weight after storage,  $w_1$  the actual weight of dry matter,  $m_1$  the initial weight of phosphorus, and  $n_1$  the actual weight of phosphorus. For example, with the 112-hour shelled sample stored at 25° C (table 2), the result for peas was  $r_1 = 45.4 \times \frac{0.300}{0.302} = 45.1$  grams; by using a similar equation and the data from the corresponding pods from table 2, the dry weight for the pods after storage was  $r_2 = 36.9 \times \frac{0.105}{0.102} = 38.0$  grams.

These values and their sum are recorded in column 6, table 2. The remaining values in column 6, tables 2 and 4, were calculated similarly. On account of the fact, however, that the actual dry weights,  $w_1$  and  $w_2$ , include a weight of dry matter which resulted from translocation during the preparatory period, and for which an accurate correction cannot be made because the phosphorus and dry matter were probably not translocated in the same ratio as present initially in the peas or pods, certain of the values of  $r_1$  and  $r_2$  may be in error by from 1 to 1½ per cent, comparable with that in the reference element. Likewise the results of all other calculations based on  $w_1$  and  $w_2$  would possess a similar error.

*Decrease in Weight of Dry Matter in Shelled Series.*—If the results for  $r_1$  or  $r_2$  be subtracted from the values  $b_1$  or  $b_2$ , the losses in weight of dry matter which occurred in the sample during storage may be obtained. These losses, however, cannot be compared directly because they are from samples having different initial dry weights. Losses can only be compared when they are calculated for samples having the same initial dry weights.

*Weight of Dry Matter of Samples Having Same Initial Dry Weight, Shelled Series.*—The dry weights after storage and the losses which occurred were recalculated for all samples of peas and pods on the basis of constant initial weights of dry matter equal to the weight of dry matter in the peas and pods of the master sample. The equation used was

$$s_1 = 0.292 \times \frac{w_1}{n_1} \quad 4$$

Here  $s_1$  is the calculated final weight of dry matter for the peas of the shelled series equal in initial weight to the master sample;  $w_1$  and  $n_1$  have the same meaning as in equation 3; and the constant 0.292 is the weight of phosphorus in the peas of the master sample. Using the data for the shelled 112-hour storage sample of peas (table 2) gave a value for  $s_1$  equal to  $45.4 \times \frac{0.292}{0.302} = 43.9$  grams and for the corresponding pods a value for  $s_2$  of  $36.9 \times \frac{0.102}{0.102} = 36.9$  grams. The same values for  $s_1$  would be obtained by using  $m_1$  in place of  $n_1$ , and  $r_1$  in place of  $w_1$ , where  $m_1$  and  $r_1$  have the same meaning as in equation 3. The results thus obtained represent the weights of dry matter after storage for shelled peas and pods from samples containing initially 49.9 grams and 43.0 grams of dry matter respectively. Similar calculations were made for all the samples of the shelled series, and the results are recorded in column 7 of tables 2 and 4. The sums of the corresponding weights of peas and pods are given in the same tables.

*Decrease in Weight of Dry Matter of Samples Having Same Initial Dry Weight, Shelled Series.*—The losses,  $l_1$ , for peas were calculated for the storage samples by subtracting the weights of dry matter for peas  $s_1$  given in column 7, tables 2 and 4, in each case from the weight (49.9 grams) of dry matter for peas in the master sample according to the equation

$$l_1 = 49.9 - s_1$$

5

The losses  $l_2$  for pods were similarly calculated. These differences, together with the over-all losses from corresponding samples, are recorded in column 8 of tables 2 and 4.

*Calculation of Final Results for the Unshelled Series.*—The weights of phosphorus present initially in the peas and pods of the unshelled storage samples were calculated by the same method as that used for the shelled series. The magnitude of the correction  $e$ , obtained with equation 1, and the initial weight of phosphorus for each sample are recorded in columns 7 and 8 respectively, in table 1. By comparison of the values of  $e$  for the actual samples given in columns 3 and 7 of table 1, the weight of phosphorus translocated during storage from pods to peas in the unshelled series is shown to be considerably greater than the amounts translocated during the preparation of the samples of the shelled series.

The weights of dry matter initially present in the peas and pods were calculated according to equation 2, and the results are given in column 5 of tables 3 and 5.

The weights of dry matter after storage from samples containing peas whose dry weights  $v_1$  were initially equal to that of the master sample were calculated from the initial weight of phosphorus  $m_1$ , the actual weight of dry matter determined for the sample  $w_1$ , and the weight of phosphorus in the master sample 0.292 gram. The equation used was

$$v_1 = 0.292 \times \frac{w_1}{m_1} \quad 6$$

The equation is based on the assumption that, for unshelled samples differing by only a small weight of initial dry matter, the changes in dry matter occurring during identical storage conditions will produce changes in actual dry weights and also translocations of phosphorus proportional to the initial dry weights of the samples or to the initial phosphorus content. Substituting the data given in table 3 for the peas from the unshelled sample stored 111 hours gave  $57.9 \times \frac{0.292}{0.297} = 56.9$

grams, and by similar calculation the weight of dry matter  $v_2$  for the corresponding pods after storage was found to be 24.6 grams. Similar calculations were made for all the remaining samples of the unshelled series, and the results are tabulated in column 6, tables 3 and 5.

The changes in weight of dry matter  $d_1$  in the peas were calculated by subtracting the weight of dry matter in the master sample (49.9 grams) from each of the values  $v_1$  given for peas in column 6, tables 3 and 5, according to the equation  $d_1 = v_1 - 49.9$ . The differences are shown in column 7, tables 3 and 5. By a similar calculation the changes in weight of dry matter  $d_2$  of the pods were obtained by subtracting the weight of the dry matter in the master sample (43.0 grams) from each of the dry weights of the pods  $v_2$ . These results, and their sums are given in column 7, tables 3 and 5.

*Calculation of the Weight of Phosphorus Translocated in Unshelled Samples of the Same Initial Weight of Dry Matter.*—The weight of phosphorus present in peas of the unshelled series after storage was calculated by use of the equation

$$p_1 = 0.292 \times \frac{n_1}{m_1} \quad 7$$

In this equation  $p_1$  represents the weight of phosphorus in peas after storage, 0.292 is the weight of phosphorus in the master sample of peas, and  $n_1$  and  $m_1$  have same meaning as in equations 1, 2, and 3. A similar calculation for  $p_2$  was made for the pods. The values for  $p_1$  and  $p_2$  are recorded in column 7 of table 11.

The changes in weight of phosphorus in the unshelled peas ( $c_1$ ) that

occurred during storage was obtained by subtracting the weight of phosphorus, 0.292 gram, in the master sample of peas from the values of  $p_1$ , as

$$c_1 = p_1 - 0.292 \quad 8$$

Similarly the changes in weight of phosphorus ( $c_2$ ) in the pods was calculated. The differences are recorded in column 8 of table 11.

*Calculation of the Weights of Some Constituents in the Storage Samples.*—The calculated weights of dry matter ( $s$  and  $v$ ) given for the shelled peas and pods in column 7 of tables 2 and 4 and for unshelled peas and pods in column 6 of tables 3 and 5 were multiplied by the corresponding percentages of the various constituents (given in cols. 2 and 6, tables 6 to 12), and the quotients divided by 100. The results appear in columns 3 and 7 of tables 6 to 12 inclusive.

## RESULTS

With each constituent determined in these tests, results are considered separately for shelled and unshelled samples of peas and of pods at each storage temperature.

*Dry Matter.*—In shelled peas stored at 25° C there was a rapid loss in weight of dry matter (table 2, col. 8), which after 112 hours amounted to 12 per cent of the original dry weight. No doubt this decrease was caused mainly by loss of certain materials in respiration, but to some extent also by the condensation of sugars to polysaccharides. The decrease of dry matter in the pods amounts to 14.2 per cent after 112 hours, the rate of loss being somewhat higher than for the peas. When the fruit was stored *unshelled*, material was rapidly translocated from pods to peas (table 3, col. 7). Instead of decreasing, the weight of dry matter of the peas increased. The translocation rate, being greater than the respiratory rate, caused an actual increase in weight of dry matter. It was not difficult to calculate an approximate value for the amount of material translocated. A loss of 18.4 grams (table 3, col. 7) of dry matter occurred in the pods stored unshelled for 111 hours. If 6.1 grams (table 2, col. 8) of this loss is assumed to be the result of respiration, then the additional loss of 12.3 grams from the pods must have been translocated. Or the amount of translocation can be determined from the changes in weight of dry matter of the peas. Respiration caused a loss of about 6.0 grams after 112 hours (table 2, col. 8). Not only was this loss balanced by translocation of material from the pods, but there accumulated a surplus of 6.8 grams above the amount present in the initial sample. This makes a total of 12.8 grams of dry matter that was translocated—a

figure which is in fair agreement with 12.3 grams, the amount found to have been translocated when the pods were used for the calculation.

When *shelled* peas and pods were stored at  $3\frac{1}{2}^{\circ}$  C, the same trend in the dry-weight curves was evident as occurred at  $25^{\circ}$ , except that the rate of loss was much slower (table 4, col. 8). The over-all loss in weight of dry matter at  $3\frac{1}{2}^{\circ}$  was 4 per cent after 188 hours; at  $25^{\circ}$ , 13.2 per cent after only 112 hours. When the fruit was stored *unshelled*, there was only a very slight increase in the weight of the dry matter of the peas (table 5, col. 7); that is, a little more dry matter moved to the peas than was lost from them by respiration. The amount of dry matter translocated can be approximately calculated as was done for the  $25^{\circ}$  C samples. The dry-weight loss of the peas from respiration after 188 hours was about 2.3 grams (table 4, col. 8). This loss was balanced by translocation; and there was, in addition, a surplus of 0.9 gram over the check, so that a total of 3.2 grams was translocated in 187 hours. If the pods are used to calculate the transfer of dry matter, the result is 3.6 grams. The amount translocated at  $3\frac{1}{2}^{\circ}$  C was about one-fourth that at  $25^{\circ}$  C.

*Carbon*.—The changes in weight of carbon at  $25^{\circ}$  C closely paralleled the dry-weight changes (table 6). The carbon loss, no doubt, was almost entirely through respiration rather than volatilization. *Shelled* peas stored for 112 hours gave a loss of 2.7 grams of carbon, but there was very little change in percentage because the average rate of loss of other dry-matter constituents kept the ratio of dry matter to carbon about constant. The changes in percentage of carbon in the pods stored shelled are probably not significant. A decrease of 2.3 grams in weight of carbon after 112 hours of storage was no doubt caused almost entirely by respiration, the average rate of loss being about the same as that in the peas. From the pods, 12.5 per cent of the carbon was lost after 112 hours; from the peas, 12.4 per cent. For the *unshelled* peas stored at  $25^{\circ}$  C the percentage of carbon varied but little over a period of 111 hours. This means that materials other than carbon accumulated in the peas at about the same rate as the carbon. Although the percentage remained constant, there was an increase in weight of 2.9 grams. In the unshelled pods the percentage of carbon remained nearly constant during storage, but 8.1 grams was lost by respiration and translocation. The over-all loss of carbon was about the same for the shelled and the unshelled: the former lost 5.0 grams and the latter 5.2 grams during the storage period.

At  $3\frac{1}{2}^{\circ}$  C the percentage of carbon in the *shelled* peas remained fairly constant, but after 188 hours there was a loss in weight of 0.8

gram, or about 3.68 per cent of the total (table 6). In the pods the percentage of carbon also remained fairly constant, but there was a loss of 0.4 gram in weight. In the *unshelled* peas there appeared to be a

TABLE 6  
CARBON IN PEAS AND PODS

Part of fruit	Hours in storage	Carbon, per cent	Weight of carbon, grams		Hours in storage	Carbon, per cent	Weight of carbon, grams	
			Corrected, after storage	Loss, during storage			Corrected, after storage	Changes during storage
	1	2	3	4	5	6	7	8
Shelled series 25° C					Unshelled series 25° C			
Peas	{ 0	43.7	21.8	0.0	0	43.7	21.8	0.0
	22	44.1	20.9	0.9	20	43.9	21.6	-0.2
	70	43.5	19.8	2.0	65	43.8	23.7	+1.9
	112	43.6	19.1	2.7	111	43.6	24.7	+2.9
Pods	{ 0	42.9	18.4	0.0	0	42.9	18.4	0.0
	22	44.2	18.1	0.3	20	43.3	17.4	-1.0
	70	43.6	17.2	1.2	65	42.8	12.7	-5.7
	112	43.5	16.1	2.3	111	42.0	10.3	-8.1
Peas and pods	{ 0	....	40.2	0.0	0	....	40.2	0.0
	22	....	39.0	1.2	20	....	39.0	-1.2
	70	....	37.0	3.2	65	....	38.4	-3.8
	112	....	35.2	5.0	111	....	35.0	-5.2
Shelled series 31.2° C					Unshelled series 31.2° C			
Peas	{ 0	43.7	21.8	0.0	0	43.7	21.8	0.0
	46	44.3	21.7	0.1	46	44.6	22.3	+0.5
	95	44.5	21.5	0.3	93	44.2	22.3	+0.5
	143	44.2	21.0	0.8	141	44.9	22.9	+1.1
	188	44.1	21.0	0.8	187	44.5	22.6	+0.8
Pods	{ 0	42.9	18.4	0.0	0	42.9	18.5	0.0
	46	43.6	18.2	0.2	46	43.2	17.5	-1.0
	95	43.8	18.2	0.2	93	43.6	17.4	-1.1
	143	43.6	18.3	0.1	141	43.4	16.6	-1.9
	188	43.3	18.0	0.4	187	43.2	16.4	-2.1
Peas and pods	{ 0	....	40.2	0.0	0	....	40.3	0.0
	46	....	39.9	0.3	46	....	39.8	-0.5
	95	....	39.7	0.5	93	....	39.7	-0.6
	143	....	39.3	0.9	141	....	39.5	-0.8
	188	....	39.0	1.2	187	....	39.0	-1.3

slight increase in the percentage of carbon, accompanied by an actual increase in weight of carbon of 0.8 gram. In the pods the percentage of carbon remained fairly constant, but there was an actual loss of 2.1 grams because of respiration and translocation. The over-all loss of

carbon was about the same in both the shelled and unshelled lots, being 1.2 grams for the former and 1.3 grams for the latter.

*Total Sugars.*—A rapid decrease occurred in the percentage of total

TABLE 7  
TOTAL SUGARS IN PEAS AND PODS

Part of fruit	Hours in storage	Total sugars, per cent	Weight of total sugars, grams		Hours in storage	Total sugars, per cent	Weight of total sugars, grams	
			Corrected after storage	Loss during storage			Corrected after storage	Loss during storage
			1	2	3	4	5	6
Shelled series 25° C						Unshelled series 25° C		
Peas	0	25.60	12.8	0.0	0	25.60	12.8	0.0
	22	15.24	7.2	5.6	20	18.40	9.1	3.7
	70	3.66	1.7	11.1	65	8.78	4.8	8.0
	112	3.38	1.5	11.3	111	5.00	2.8	10.0
Pods	0	28.7	12.3	0.0	0	28.70	12.4	0.0
	22	27.0	11.1	1.2	20	28.00	11.3	1.1
	70	22.8	9.0	3.3	65	16.20	4.8	7.6
	112	18.4	6.8	5.5	111	5.89	1.5	10.9
Peas and pods	0	....	25.1	0.0	0	....	25.2	0.0
	22	....	18.3	6.8	20	....	20.4	4.8
	70	....	10.7	14.4	65	....	9.6	15.6
	112	....	8.3	16.8	111	....	4.3	20.9
Shelled series 3½° C						Unshelled series 3½° C		
Peas	0	25.6	12.8	0.0	0	25.6	12.8	0.0
	46	23.3	11.4	1.4	46	23.3	11.7	1.1
	95	21.9	10.6	2.2	93	24.2	12.2	0.6
	143	17.8	8.5	4.3	141	22.7	11.6	1.2
	188	16.3	7.8	5.0	187	22.6	11.5	1.3
Pods	0	28.7	12.3	0.0	0	28.7	12.4	0.0
	46	29.2	12.2	0.1	46	27.4	11.1	1.3
	95	28.7	11.9	0.4	93	26.6	10.2	2.2
	143	28.0	11.8	0.5	141	23.7	9.1	3.3
	188	26.2	10.9	1.4	187	22.5	8.6	3.8
Peas and pods	0	....	25.1	0.0	0	....	25.2	0.0
	46	....	23.6	1.5	46	....	22.8	2.4
	95	....	22.5	2.6	93	....	22.4	2.8
	143	....	20.3	4.8	141	....	20.7	4.5
	188	....	18.7	6.4	187	....	20.1	5.1

sugars in the *shelled* peas stored at 25° C (table 7). The weight of total sugars also decreased rapidly; of the initial amount only 11.6 per cent remained after 112 hours. Jones and Bisson<sup>(a)</sup> and Boswell<sup>(a)</sup> have shown that the sugar in the peas reacts like sucrose during the analysis. The

loss of total dry weight in shelled peas was 6.00 grams; that of the total sugars, 11.3 grams. As the decrease in the total sugars exceeded that in the dry weight by 5.3 grams, the loss of sugars can be caused only partly by respiration; there must therefore be a transformation into other substances. In pods stored shelled there was a decrease in the percentage as well as the weight of sugar. As the dry-weight loss was 6.1 grams (table 2) and that of the total sugars 5.6 grams, evidently compounds other than sugar—perhaps starch and other polysaccharides—are hydrolyzed to sugar and used in respiration. Peas stored *unshelled* had a higher percentage as well as a greater weight of sugars than those shelled for each of the periods, a phenomenon that can be explained by the translocation of sugars from the pods. In the pods the percentage as well as the weight of total sugars decreased very rapidly. In the shelled pods 45 per cent of the initial amount of sugar was lost after 112 hours; in the unshelled, 88 per cent.

In peas and pods stored *shelled* at  $3\frac{1}{2}^{\circ}$  C, there was a decrease in the percentage as well as in the weight of total sugars (table 7). The shelled peas lost sugar more rapidly than the shelled pods. In the former, 61 per cent of the total sugar still remained after 188 hours of storage as compared with 89.0 per cent in the latter. In the *unshelled* peas and pods there was also a decrease in the percentage and weight of total sugars.

*Starch.*—In the *shelled* peas stored at  $25^{\circ}$  C both the percentage and the weight of starch increased until the third day; thereafter a decrease occurred (table 8). In the shelled pods there was a decrease during the entire storage period in both the percentage and weight of starch. In the *unshelled* peas there was an increase, which was considerably higher than in the shelled, and continued to the end of the storage period, no doubt because of the translocation and condensation of sugars. In the unshelled pods the decrease in percentage and weight of starch was about the same as in the shelled.

In the *shelled* peas stored at  $3\frac{1}{2}^{\circ}$  C (table 8) there was a very slight increase in the percentage as well as in the weight of starch, whereas in the shelled pods there was some decrease. In the *unshelled* peas and pods the same trends occurred as in the shelled.

Boswell<sup>10</sup> showed that the most pronounced change occurring in the maturation of peas on the vine was the increase in the percentage of starch and other polysaccharides. Bisson and Jones,<sup>11</sup> studying the changes occurring in the fruit during development, found that fruit left on the vine after the best marketable stage showed a considerable reduction in percentage as well as absolute weight of sucrose for a con-

siderable time and, during this same period, a pronounced increase in the percentage as well as the absolute weight of starch and other acid-hydrolyzable polysaccharides.

TABLE 8  
STARCH IN PEAS AND PODS

Part of fruit	Hours in storage	Starch, per cent	Weight of starch, grams		Hours in storage	Starch, per cent	Weight of starch, grams	
			Corrected after storage	Changes during storage			Corrected after storage	Changes during storage
			<i>I</i>	<i>S</i>	<i>S</i>	<i>4</i>	<i>5</i>	<i>6</i>
Shelled series 25° C								
Peas	0	14.5	7.24	0.00	0	14.5	7.24	0.00
	22	18.5	8.77	+1.53	20	18.3	9.02	+1.78
	70	23.4	10.62	+3.38	65	23.3	12.03	+5.39
	112	20.6	9.04	+1.80	111	22.7	12.87	+5.63
Unshelled series 25° C								
Pods	0	5.26	2.26	0.00	0	5.26	2.26	0.00
	22	3.44	1.41	-0.85	20	4.24	1.70	-0.56
	70	1.42	0.56	-1.70	65	1.98	0.58	-1.68
	112	1.05	0.39	-1.87	111	1.21	0.30	-1.96
Peas and pods	0	.....	9.50	0.00	0	.....	9.50	0.00
	22	.....	10.18	+0.68	20	.....	10.72	+1.22
	70	.....	11.18	+1.68	65	.....	13.21	+3.71
	112	.....	9.43	-0.07	111	.....	13.17	+3.67
Shelled series 3½° C								
Peas	0	14.5	7.24	0.00	0	14.5	7.24	0.00
	46	15.2	7.45	+0.21	46	14.8	7.40	+0.16
	95	14.5	7.02	-0.22	93	14.8	7.46	+0.22
	143	17.0	8.06	+0.85	141	14.9	7.58	+0.34
	188	16.4	7.81	+0.57	187	15.3	7.72	+0.48
Pods	0	5.26	2.26	0.00	0	5.26	2.26	0.00
	46	4.52	1.89	-0.37	46	4.42	1.80	-0.46
	95	4.14	1.72	-0.54	93	4.30	1.75	-0.51
	143	3.99	1.68	-0.58	141	4.11	1.87	-0.69
	188	4.11	1.71	-0.55	187	4.27	1.82	-0.64
Peas and pods	0	.....	9.50	0.00	0	.....	9.50	0.00
	46	.....	9.34	-0.16	46	.....	9.20	-0.30
	95	.....	8.74	-0.76	93	.....	9.21	-0.29
	143	.....	9.77	+0.27	141	.....	9.15	-0.35
	188	.....	9.52	+0.02	187	.....	9.34	-0.16

The data at hand show that fruit harvested from the vine at edible maturity and stored at 25° C underwent the same changes for a time as fruit attached to the vine. The soluble solids moving from the pods to the peas are chiefly sugars, some of which are condensed to form higher

polysaccharides, producing a definite increase in the percentage as well as the weight of starch. Condensation occurred in the shelled as well as the unshelled peas except that in the former there was no translocation

TABLE 9  
CRUDE FIBER IN PEAS AND PODS

Part of fruit	Hours in storage	Crude fiber, per cent	Weight of crude fiber, grams		Hours in storage	Crude fiber, per cent	Weight of crude fiber, grams	
			Corrected after storage	Changes during storage			Corrected after storage	Changes during storage
			1	2	3	4	5	6
Shelled series 25° C								
Peas	0	8.63	4.31	0.00	0	8.63	4.31	0.00
	22	9.83	4.66	+0.35	20	9.67	4.77	+0.46
	70	10.74	4.88	+0.57	65	9.91	5.37	+1.06
	112	11.25	4.94	+0.63	111	9.60	5.44	+1.13
Unshelled series 25° C								
Pods	0	18.1	7.78	0.00	0	18.1	7.78	0.00
	22	19.3	7.91	+0.13	20	19.2	7.72	-0.06
	70	20.9	8.23	+0.45	65	25.8	7.61	-0.17
	112	22.4	8.27	+0.49	111	31.2	7.68	-0.10
Peas and pods	0	....	12.09	0.00	0	....	12.09	0.00
	22	....	12.57	+0.48	20	....	12.49	+0.40
	70	....	13.11	+1.02	65	....	12.98	+0.89
	112	....	13.21	+1.12	111	....	13.12	+1.03
Shelled series 3½° C								
Peas	0	8.63	4.31	0.00	0	8.63	4.31	0.00
	46	9.15	4.48	+0.17	46	9.02	4.51	+0.20
	95	9.52	4.61	+0.30	93	8.99	4.53	+0.22
	143	9.85	4.69	+0.38	141	8.97	4.57	+0.26
	188	9.85	4.69	+0.38	187	9.15	4.65	+0.34
Pods	0	18.1	7.78	0.00	0	18.1	7.78	0.00
	46	18.2	7.61	-0.17	46	18.9	7.67	-0.11
	95	18.5	7.68	-0.10	93	19.9	7.92	+0.14
	143	18.7	7.85	+0.07	141	20.6	7.87	+0.09
	188	19.1	7.95	+0.17	187	20.8	7.90	+0.12
Peas and pods	0	....	12.09	0.00	0	....	12.09	0.00
	46	....	12.09	0.00	46	....	12.18	+0.09
	95	....	12.29	+0.20	93	....	12.45	+0.36
	143	....	12.54	+0.45	141	....	12.44	+0.35
	188	....	12.04	+0.55	187	....	12.55	+0.46

and therefore less condensation. As these data show, peas do not lose their power to synthesize starch even though the fruit has been severed from the vine or the peas removed from the pod. These results are not strictly in accord with those of Kertesz<sup>(6)</sup> who found no increase of starch in green shelled peas.

*Crude Fiber.*—The *shelled* peas stored at 25° C showed an increase in both the percentage and the weight of crude fiber (table 9). The percentage increase was more marked because of loss in dry weight from respiration. The percentage and weight of crude fiber in the pods also increased, because of loss in dry matter rather than because of any large increase in the weight of crude fiber. In the *unshelled* peas the increase in percentage of crude fiber is less than in the shelled because translocation increases the actual weight of dry matter. For the same storage period the percentage was always less in the unshelled peas even though the weight of crude fiber was actually greater. The unshelled pods gave the most marked increase in the percentage of crude fiber, though the actual weight of crude fiber remained nearly constant. This pronounced increase in percentage was caused by dry-weight losses in translocation and respiration. These percentage data would be rather misleading as to the actual changes that occurred if the weight data were not also considered.

In the *shelled* peas stored at 3½° C the percentage as well as the weight of crude fiber increased slightly (table 9). In the shelled pods the increase in percentage and weight of fiber was even less than in the peas. In the *unshelled* peas the percentage increase in the fiber was less than in the shelled because of the influx of material from the pods; the weight increase was about the same as for the shelled. The unshelled pods showed an increase in percentage of fiber, caused chiefly by a loss of materials other than crude fiber. The increase in weight was about the same as for the shelled pods.

*Nitrogen.*—In both the peas and pods stored *shelled* at 25° C there was a slight increase in the percentage of nitrogen, caused no doubt by the decreasing weight of dry matter (table 10). There was a small decrease in the weight of nitrogen. In the *unshelled* peas both the percentage and weight of nitrogen increased slightly; the influx of nitrogenous material was sufficiently rapid to decrease the ratio of dry matter to nitrogen, thereby causing an increase in nitrogen percentage. The decreasing percentage of nitrogen in the unshelled pods means that there was a more rapid loss of nitrogen than of dry matter, thereby causing an increase in the ratio of dry matter to nitrogen. More than one-half of the nitrogen present in the initial sample of pods was lost in 111 hours. The over-all loss of nitrogen from the entire system must represent losses to the atmosphere in some form and was about the same for the shelled and unshelled material.

In both peas and pods stored *shelled* at 3½° C there was very little change in the percentage of nitrogen after 188 hours (table 10), because

the amount of dry matter lost was so slight that it hardly affected the ratio of dry matter to nitrogen. Here, again, there seems to have been a slight loss of nitrogen to the atmosphere. In the unshelled peas stored

TABLE 10  
NITROGEN IN PEAS AND PODS

Part of fruit	Hours in storage	Nitrogen, per cent	Weight of nitrogen, grams		Hours in storage	Nitrogen, per cent	Weight of nitrogen, grams	
			Corrected after storage	Changes during storage			Corrected after storage	Changes during storage
	1	2	3	4	5	6	7	8
Shelled series 25° C					Unshelled series 25° C			
Peas	0	4.79	2.39	0.00	0	4.79	2.39	0.00
	22	4.93	2.34	-0.05	20	4.94	2.43	+0.04
	70	5.13	2.32	-0.07	65	4.97	2.69	+0.30
	112	5.28	2.32	-0.07	111	5.05	2.86	+0.47
Pods	0	2.43	1.04	0.00	0	2.43	1.04	0.00
	22	2.47	1.01	-0.03	20	2.41	0.97	-0.07
	70	2.59	1.02	-0.02	65	2.23	0.66	-0.38
	112	2.67	0.99	-0.05	111	1.88	0.46	-0.58
Peas and pods	0	....	3.43	0.00	0	....	3.43	0.00
	22	....	3.35	-0.08	20	....	3.40	-0.03
	70	....	3.34	-0.09	65	....	3.35	-0.08
	112	....	3.31	-0.12	111	....	3.32	-0.11
Shelled series 3½° C					Unshelled series 3½° C			
Peas	0	4.79	2.39	0.00	0	4.79	2.39	0.00
	46	4.76	2.33	-0.06	46	4.67	2.34	-0.05
	95	4.83	2.34	-0.05	93	4.65	2.34	-0.05
	143	4.96	2.36	-0.03	141	4.64	2.36	-0.03
	188	4.93	2.35	-0.04	187	4.58	2.33	-0.06
Pods	0	2.43	1.04	0.00	0	2.43	1.04	0.00
	46	2.54	1.06	+0.02	46	2.38	0.97	-0.07
	95	2.39	0.99	-0.05	93	2.39	0.95	-0.09
	143	2.44	1.02	-0.02	141	2.48	0.95	-0.09
	188	2.41	1.00	-0.04	187	2.52	0.96	-0.08
Peas and pods	0	....	3.43	0.00	0	....	3.43	0.00
	46	....	3.39	-0.04	46	....	3.31	-0.12
	95	....	3.33	-0.10	93	....	3.29	-0.14
	143	....	3.38	-0.05	141	....	3.31	-0.12
	188	....	3.35	-0.08	187	....	3.29	-0.14

at 3½° C there appears to have been a slight decrease in the percentage of nitrogen, and little or no change in its weight, a condition indicating that very little or no translocation of nitrogenous constituents occurred at this temperature. The decrease in percentage was probably caused by

TABLE 11  
PHOSPHORUS IN PEAS AND PODS

Part of fruit	Hours in storage	Phosphorus, per cent	Weight of phosphorus, grams		Hours in storage	Phosphorus, per cent	Weight of phosphorus, grams	
			Corrected after storage	Changes during storage			Corrected after storage	Changes during storage
			1	2			3	4
Shelled series 25° C:					Unshelled series 25° C			
Peas	0	0.586	<i>p</i> <sub>1</sub> 0.292	<i>c</i> <sub>1</sub> 0.000	0	0.586	<i>p</i> <sub>1</sub> 0.292	<i>c</i> <sub>1</sub> 0.000
	22	0.617	0.292	0.000	20	0.610	0.301	+0.009
	70	0.644	0.292	0.000	65	0.615	0.333	+0.041
	112	0.666	0.292	0.000	111	0.628	0.357	+0.065
Pods	0	0.238	<i>p</i> <sub>2</sub> 0.102	<i>c</i> <sub>2</sub> 0.000	0	0.238	<i>p</i> <sub>2</sub> 0.102	<i>c</i> <sub>2</sub> 0.000
	22	0.248	0.102	0.000	20	0.232	0.093	-0.009
	70	0.258	0.102	0.000	65	0.206	0.061	-0.041
	112	0.277	0.102	0.000	111	0.152	0.037	-0.065
Peas and pods	0	.....	0.394	0.000	0	.....	0.394	0.000
	22	.....	0.394	0.000	20	.....	0.394	0.000
	70	.....	0.394	0.000	65	.....	0.394	0.000
	112	.....	0.394	0.000	111	.....	0.394	0.000
Shelled series 31½° C:					Unshelled series 31½° C:			
Peas	0	0.586	<i>p</i> <sub>1</sub> 0.292	<i>c</i> <sub>1</sub> 0.000	0	0.586	<i>p</i> <sub>1</sub> 0.292	<i>c</i> <sub>1</sub> 0.000
	46	0.596	0.292	0.000	46	0.594	0.297	+0.005
	95	0.603	0.292	0.000	93	0.595	0.300	+0.008
	143	0.613	0.292	0.000	141	0.593	0.302	+0.010
	188	0.614	0.292	0.000	187	0.594	0.302	+0.010
Pods	0	0.238	<i>p</i> <sub>2</sub> 0.102	<i>c</i> <sub>2</sub> 0.000	0	0.238	<i>p</i> <sub>2</sub> 0.102	<i>c</i> <sub>2</sub> 0.000
	46	0.244	0.102	0.000	46	0.240	0.097	-0.005
	95	0.245	0.102	0.000	93	0.237	0.094	-0.008
	143	0.244	0.102	0.000	141	0.241	0.092	-0.010
	188	0.244	0.102	0.000	187	0.242	0.092	-0.010
Peas and pods	0	.....	0.394	0.000	0	.....	0.394	0.000
	46	.....	0.394	0.000	46	.....	0.394	0.000
	95	.....	0.394	0.000	93	.....	0.394	0.000
	143	.....	0.394	0.000	141	.....	0.394	0.000
	188	.....	0.394	0.000	187	.....	0.394	0.000

an influx of dry matter together with an actual loss in weight of nitrogen. The pods showed a slight increase in the percentage but a decrease in the weight of nitrogen. The increase in percentage must be explained by a loss in weight of dry matter. The over-all loss of nitrogen appears to have been higher from the unshelled than from the shelled material.

*Phosphorus.*—In both peas and pods stored *shelled* at 25° C there was

an increase in the percentage of phosphorus because the respiratory losses decreased the ratio of dry matter to phosphorus (table 11). The corrected weight of phosphorus for the peas has been kept constant

TABLE 12  
MAGNESIUM IN PEAS AND PODS

Part of fruit	Hours in storage	Magnesium, per cent	Weight of magnesium, grams		Hours in storage	Magnesium, per cent	Weight of magnesium, grams	
			Corrected after storage	Changes during storage			Corrected after storage	Changes during storage
			1	2	3	4	5	6
Shelled series 25° C					Unshelled series 25° C			
Peas	0	0.164	0.082	0.000	0	0.164	0.082	0.000
	22	0.167	0.079	-0.003	20	0.168	0.083	+0.001
	70	0.175	0.079	-0.003	65	0.170	0.092	+0.010
	112	0.179	0.079	-0.003	111	0.176	0.100	+0.018
Pods	0	0.481	0.207	0.000	0	0.481	0.207	0.000
	22	0.495	0.203	-0.004	20	0.509	0.205	-0.002
	70	0.524	0.206	-0.001	65	0.618	0.182	-0.025
	112	0.564	0.208	+0.001	111	0.729	0.180	-0.027
Peas and pods	0	.....	0.289	0.000	0	.....	0.289	0.000
	22	.....	0.282	-0.007	20	.....	0.288	-0.001
	70	.....	0.285	-0.004	65	.....	0.274	-0.015
	112	.....	0.287	-0.002	111	.....	0.280	-0.009
Shelled series 3½° C					Unshelled series 3½° C			
Peas	0	0.164	0.082	0.000	0	0.164	0.082	0.000
	46	0.163	0.080	-0.002	46	0.162	0.081	-0.001
	95	0.164	0.079	-0.003	93	0.161	0.081	-0.001
	143	0.167	0.079	-0.003	141	0.161	0.082	0.000
	188	0.169	0.080	-0.002	187	0.162	0.082	0.000
Pods	0	0.481	0.207	0.000	0	0.481	0.207	0.000
	46	0.497	0.208	+0.001	46	0.504	0.205	-0.002
	95	0.497	0.206	-0.001	93	0.501	0.200	-0.007
	143	0.511	0.215	+0.008	141	0.508	0.194	-0.013
	188	0.516	0.215	+0.008	187	0.530	0.201	-0.006
Peas and pods	0	.....	0.289	0.000	0	.....	0.289	0.000
	46	.....	0.288	-0.001	46	.....	0.287	-0.003
	95	.....	0.285	-0.004	93	.....	0.281	-0.008
	143	.....	0.294	+0.005	141	.....	0.276	-0.013
	188	.....	0.295	+0.006	187	.....	0.283	-0.006

throughout because we have assumed in the calculations that the check and stored samples had the same initial weight of phosphorus, associated initially with a constant amount of dry matter. In the unshelled peas there was a considerable increase in the weight of phosphorus. The rate

of increase in percentage of phosphorus was greater than the rate of increase in percentage of dry matter and this caused a decrease in the ratio of dry matter to phosphorus and therefore an increase in phosphorus percentage. In the pods there was a considerable decrease in the percentage of phosphorus; evidently, therefore, the rate of decrease in percentage of phosphorus-containing constituents was greater than that of other dry-matter constituents. A large fraction of the phosphorus in the pods was translocated, more than half of this element by weight being moved out in 111 hours.

In both the peas and pods stored *shelled* at  $3\frac{1}{2}$ ° C the weight of phosphorus remained constant (table 11). Respiration caused a loss in weight of dry matter, thus decreasing the ratio of dry matter to phosphorus and increasing the percentage of phosphorus. In the *unshelled* peas there was an increase in both percentage and weight of phosphorus. In the unshelled pods the percentage of phosphorus changed very little despite a decreased weight of phosphorus. After 187 hours at  $3\frac{1}{2}$ ° C, 10 per cent of the phosphorus in the pods had been translocated; after 111 hours at 25° C, 65 per cent.

**Magnesium.**—In both peas and pods stored *shelled* at 25° C there was an increase in the percentage of magnesium, the weight of magnesium remaining constant (table 12). In the *unshelled* peas there was a slight increase in the percentage. If both the magnesium and the dry matter had been translocated during storage at such rates that their initial ratio was maintained, then of course the percentage would have remained constant. In the unshelled pods the percentage of magnesium also increased, even though this element was translocated in determinable amounts to the peas. The increase in percentage indicates that the dry matter is lost from the pods sufficiently fast to cause a rapid decrease in the ratio of dry matter to magnesium. The percentage changes in phosphorus and magnesium in the unshelled pods showed considerable contrasts at this temperature. The decrease in the percentage of phosphorus and the increase in the percentage of magnesium indicate that the former is readily translocated, whereas the latter is not.

In both peas and pods stored *shelled* at  $3\frac{1}{2}$ ° C, there was a slight increase in the percentage of magnesium (table 12). Though the weight of magnesium should be constant in both pods and peas, in the former there was some fluctuation. In the *unshelled* peas both the percentage and the weight of magnesium remained almost constant; no significant movement of magnesium was discerned at this temperature. In the unshelled pods there was an increase in the percentage of magnesium, due to the decrease in weight of dry matter.

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# H I L G A R D I A

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## BIOLOGY OF THE MEALY PLUM APHID, HYALOPTERUS PRUNI (GEOFFROY)<sup>1</sup>

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- Hyalopterus pruni* (Geoffroy)  
*Aphis pruni* Geoffroy 1762  
*Aphis arundinis* Fabricius 1775  
*Aphis pruni* Fabricius 1775  
*Aphis amygdali* Blanchard 1840  
*Aphis prunifex* Amyot 1847  
*Aphis calamaphis* Amyot 1847  
*Aphis gracilis* Walker 1850  
*Hyalopterus pruni* (Fabr.) 1857  
*Hyalopterus arundinis* (Fabr.) 1857  
*Aphis phragmitidicola* Oestlund 1886  
*Hyadaphis umbellariae* Davidson 1911

## HISTORICAL REVIEW OF THE TAXONOMY OF HYALOPTERUS PRUNI (GEOFFROY)

THE SCIENTIFIC NAME of this species has been changed repeatedly; in fact, if each of the following authors is recognized, the status of the name has been changed twenty-one times, in the course of which the species has been described eight times as new. This review of the taxonomy does not include many of the lesser notes of an economic nature, which give *Hyalopterus arundinis* (Fabr.) priority over *H. pruni* (Fabr.) and vice versa.

1737. De Réamur<sup>(31)\*</sup> recorded certain observations on an aphid on plum trees in France. This aphid was coated with a white powder. There can be little doubt but that he was dealing with *Hyalopterus pruni* (Geoff.).
1762. *Aphis pruni* Geoffroy. This species was described in an anonymous publication, known to be the work of E. L. Geoffroy. This was the earliest use of a binomial name for this species, according to Hottes.<sup>(31)</sup>

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\* Superscript numbers in parentheses refer to "Literature Cited" at the end of this paper.

1773. *Aphis pruni* Geoffroy was recognized and cited by De Geer<sup>(19)</sup> in his observations on the life history of this species.
1794. *Aphis arundinis epigejos* Fabricius<sup>(20)</sup> and *Aphis pruni domesticae* Fabricius.<sup>(21)</sup> At this date the two species listed above were described, the former from *Arundinis epigejos* (*Calamagrostis epigejos*) and the latter from *Prunus domestica* L. Hottes and Frison<sup>(22)</sup> state that Fabricius,<sup>(20)</sup> in 1775 (a work not available to this author), described *arundinis* and *pruni*, and that the former has page priority.
1840. *Aphis amygdali* Blanchard.<sup>(23)</sup> Blanchard collected the species on peach near Paris and described it as new.
1847. *Aphis prunifex* Amyot and *Aphis calamaphis* Amyot.<sup>(24)</sup> At this time Amyot renamed a number of species of *Aphis* by adding the suffix "fex" to many of the existing species. *Aphis prunifex* was derived from *Aphis pruni* Fabr., which was listed as a synonym. In addition, he described the species as new under the name of *A. calamaphis*.
1850. *Aphis pruni* Fabr. Walker<sup>(25)</sup> recognized that *pruni* and *arundinis* of Fabricius were identical and gave the former priority. He also listed *Aphis prunifex* Amyot and *A. calamaphis* Amyot as synonyms.
1860. *Hyalopterus pruni* (Fabr.). Passerini<sup>(26)</sup> designated *Aphis pruni* Fabr. as the type of the genus *Hyalopterus* of Koch<sup>(27)</sup> since that author had failed to name a type species. *Aphis arundinis* Fabr. was listed as a synonym of *A. pruni* Fabr.
1879. *Hyalopterus pruni* (Fabr.) and *Hyalopterus arundinis* (Fabr.). Buckton<sup>(28)</sup> saw fit to separate these two species which had been united by Walker.<sup>(29)</sup> As synonyms of *H. pruni* (Fabr.) he listed *Aphis pruni* Fabr. and *Aphis pruni prunifex* Amyot. As synonyms of *H. arundinis* (Fabr.) he listed *Aphis arundinis* Fabr., *Aphis calamaphis* Amyot, and *Hyalopterus arundinis* Koch. (Koch, however, did not name a species *arundinis*.) At the same time he listed *Aphis calamaphis* Amyot and *A. prunifex* Amyot as synonyms of *Aphis pruni* Réamur [= *Anuraphis helichrysi* (Kalt.)]
1886. *Aphis phragmitidicola* Oestlund. Oestlund<sup>(30)</sup> described the species as new, under this name, although he remarked, "This may be the Linnean species *arundinis*." Linnaeus, however, did not name a species *arundinis*.
1887. *Hyalopterus arundinis* (Fabr.). Oestlund<sup>(31)</sup> recognized this name and placed his *Aphis phragmitidicola* Oestl. as a synonym. As additional synonyms he listed *Aphis arundinis* Fabr. and *Hyalopterus arundinis* Koch.
1893. *Hyalopterus pruni* (Fabr.). Osborne and Sirrine<sup>(32)</sup> recognized this name and listed *H. arundinis* (Fabr.) as a synonym.
1897. *Hyalopterus pruni* (Fabr.), *Hyalopterus arundinis* (Fabr.), and *Aphis pruni* Fabr. Lowe<sup>(33)</sup> stated that these three species attack the plum and gave notes on each species.
1901. *Hyalopterus arundinis* (Fabr.). Hunter<sup>(34)</sup> recognized this name, which is obviously a misspelling of *Hyalopterus*. He listed *Aphis pruni* Fabr. and *A. phragmitidicola* Oestl. as synonyms. He also listed *Aphis prunifoliae* Fitch as a synonym although this latter is now known to be a distinct species.
1905. *Hyalopterus*. Kirkaldy<sup>(35)</sup> listed *Hyalopterus* Hunter as a synonym of *Hyalopterus*.
1906. *Hyalopterus arundinis* Fabr. and *Hyalopterus pruni* Fabr. Schouteden<sup>(36)</sup> considered these two species as distinct, the former on *Arundo phragmites* and the latter on *Prunus* sp. He recognized *Hyalopterus phragmiticola* Oestlund as a

- synonym of *H. arundinensis* (Fabr.). This was obviously a misspelling of *phragmitidicola* Oestl.
1907. *Hyalopterus pruni* (Fabr.). Mordwilko<sup>(11)</sup> gave this species priority over *H. arundinensis* (Fabr.).
1910. *Hyalopterus arundinensis* (Fabr.). Davis<sup>(12)</sup> recognized this species and placed *phragmitidicola* Oestl. as a synonym.
1910. *Hyalopterus pruni* (Fabr.) and *Hyalopterus arundinensis* (Fabr.). Henrich<sup>(13)</sup> considered these species distinct from each other and gave a key for their separation.
1911. *Hyadaphis umbellulariae* Davidson. W. M. Davidson<sup>(14)</sup> described the species as new from alate females collected on the bay tree, *Umbellularia californica* Nutt.
1911. *Hyalopterus arundinensis* (Fabr.). Essig<sup>(15)</sup> recognized this name and listed *Aphis pruni* Fabr. and *Aphis phragmitidicola* Oestl. as synonyms.
1914. *Hyalopterus pruni* (Fabr.). Theobald<sup>(16)</sup> gave this name priority over *H. arundinensis* (Fabr.) and *H. phragmiticola* Oestl. (misspelling of *H. phragmitidicola* Oestl.).
1917. *Hyalopterus pruni* (Fabr.). Van der Goot<sup>(17)</sup> listed as synonymous of this species, the following: *Aphis pruni* Fabr., *A. arundinensis* Fabr., *Hyalopterus pruni* Koch, and *Hyalopterus arundinensis* Koch.
1917. *Hyalopterus pruni* (Fabr.). Matsumura<sup>(18)</sup> gave this name priority over the same names as van der Goot.
1918. *Hyalopterus pruni* (Fabr.). Theobald<sup>(16)</sup> placed *H. arundinensis* Fabr. and *H. phragmiticola* Oestl. (misspelling of *phragmitidicola* Oestl.) as synonyms of this name.
1918. *Hyalopterus pruni* (Fabr.). Das<sup>(19)</sup> gave this name priority over *H. arundinensis* Fabr.
1919. *Hyalopterus arundinensis* (Fabr.). W. M. Davidson<sup>(10)</sup> recognized this name, and placed *Aphis arundinensis* Fabr., *A. pruni* Fabr., *A. phragmiticola* Oestl. (misspelling of *phragmitidicola* Oestl.) and *Hyadaphis umbellulariae* Davidson as synonyms.
1919. *Hyalopterus arundinensis* (Fabr.). Arkhangelsky<sup>(20)</sup> gave this name priority over *H. pruni* (Fabr.).
1920. *Hyalopterus*. Baker<sup>(21)</sup> placed the genus *Hayhurstia* Del Guercio, 1917, as a synonym of *Hyalopterus* and suggested that *Pergandeidia* Schouteden may be a synonym, as well.
1921. *Hyalopterus pruni* (Fabr.). Takahashi<sup>(22)</sup> gave this name priority over *Aphis pruni* Fabr., *A. arundinensis* Fabr., *Hyalopterus pruni* Koch, and *H. arundinensis* Koch.
1925. *Hyalopterus arundinensis* (Fabr.). Laing<sup>(23)</sup> used this name and placed *Aphis gracilis* Walker as a synonym, after examining Walker's type.
1925. *Hyalopterus arundinensis* (Fabr.). Davidson<sup>(14)</sup> listed *H. pruni* (Fabr.) as a synonym of this species.
1927. *Hyalopterus arundinensis* (Fabr.). Theobald<sup>(16)</sup> gave this name priority and placed as synonyms: *Aphis pruni* Fabr., *A. arundinensis* Fabr., *A. calamaphis* Amyot, *A. prunifex* Amyot, *A. gracilis* Walker, *Hyalopterus pruni* Koch, *H. phragmiticola* Oestl. (misspelling of *phragmitidicola* Oestl.).
1928. *Hyalopterus pruni* (Fabr.). Opmanis<sup>(24)</sup> gave this name priority over *Aphis pruni* Fabr., *A. arundinensis* Fabr., *Hyalopterus arundinensis* (Fabr.) and *Hyalop-*

- terus phragmiticola* Oestl. (misspelling of *phragmitidicola* Oestl.). In this same publication he listed *Aphis prunifex* Amyot and *Aphis calamaphis* Amyot, as synonyms of *Brachycaudus helichrysi* Kalt. and cites Buckton as the authority for this change. These two names are now considered to be synonymous with *Hyalopterus pruni* (Geoffroy), and distinct from *B. helichrysi* Kalt.
1930. *Aphis pruni* Geoffroy. Hottes<sup>(11)</sup> called attention to Geoffroy's name *pruni* published anonymously in 1762 but known to be the work of Geoffroy. He pointed out that this use of *pruni* preceded *Aphis pruni* Scopoli 1763, *Aphis pruni* Fabr. 1775, and *Aphis pruni* Koch 1854. He therefore stated that this name should be used instead of *arundinis* Fabr. or *pruni* Fabr.
1931. *Hyalopterus arundinis* (Fabr.). Börner<sup>(12)</sup> placed *Aphis amygdali* Blanchard as a synonym of *Hyalopterus arundinis* (Fabr.).
1931. *Hyalopterus arundinis* (Fabr.). Takahashi<sup>(13)</sup> used this name and listed *arundinis* and *pruni* of Fabricius and of Koch as synonyms.
1931. *Hyalopterus pruni* (Geoffroy). Hottes and Frison<sup>(14)</sup> referred to the species under this name, and placed Geoffroy's *Aphis pruni* in the genus *Hyalopterus*. In this work *A. pruni* Fabr. and *A. arundinis* Fabr. are listed as synonyms.
1932. *Hyalopterus phragmitidicola* Oestl. Börner<sup>(15)</sup> suggested that this name be used in place of *H. pruni* (Fabr.) and *H. arundinis* (Fabr.). He pointed out that *Aphis pruni* Scopoli 1763 has precedence over *A. pruni* Fabr. 1775, so that the latter has been listed as a synonym of *A. arundinis* Fabr. Fabricius listed a single host for *Aphis arundinis*, namely, *Arundo epigejos* (= *Calamagrostis epigeios*), which, Börner pointed out, is a hard-leaved sandgrass belonging to the tribe Agrostideae and not at all similar to the known alternate hosts of the plum aphid, *Arundo* and *Phragmites*, which belong to the Festuceae. His investigations led him to the conclusion that the mealy plum aphid does not live on *Calamagrostis epigeios* and that *A. arundinis* Fabr. referred to another species. Consequently, he proposed that the name *phragmitidicola* Oestl. be used.
1932. *Hyalopterus arundinis* (Fabr.). Gillette and Palmer<sup>(16)</sup> used the above name, and listed *Aphis pruni* Fabr. as a synonym.

*Conclusion on Nomenclature.*—The very pertinent observations of Börner<sup>(15)</sup> indicate that the name *Aphis pruni* Fabr. was preoccupied and that *Aphis arundinis* Fabr. may have referred to some other species than the mealy plum aphid, since it was described on a plant on which this species could not be found. The writer's observations lend support to this view (see discussion under "Host Plants," pp. 201–202). In view of these facts, it is fortunate that Hottes<sup>(11, 12)</sup> has called attention to the priority of *Aphis pruni* Geoffroy and placed it in the genus *Hyalopterus*.

#### GEOGRAPHICAL DISTRIBUTION

*Hyalopterus pruni* (Geoff.) has been frequently reported in many sections of the world, and particularly in the north temperate zone. It has been reported in Africa (Union of South Africa), Australia, Belgium, Canada, China, Denmark, Egypt, England, France, Germany, India, Ireland, Italy, Japan, Java, Latvia, Morocco, New Zealand, Norway,

Palestine, Peru, Portugal, Russia, Scotland, Slavonia, Sweden, and Switzerland. This distribution is indicated on the map in figure 1.

In the United States this species was first reported from the vicinity of Carmel, California, in 1881. It was reported in Minnesota in 1885. At present it is known to occur in California, Colorado, Connecticut, Illinois, Iowa, Kansas, Maine, Minnesota, Nebraska, Nevada, New Jersey, New York, Ohio, South Dakota, and Utah.

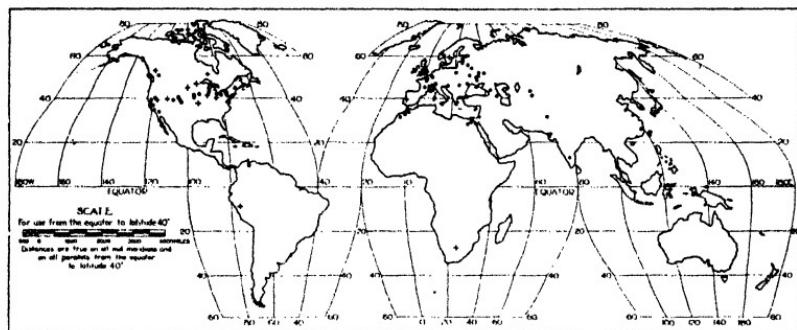


Fig. 1.—Distribution of *Hyalopterus pruni* (Geoff.) in the world. A dot indicates a definite locality; a cross indicates an indefinite locality, as a country or state. (Outline map adapted from Goode's series of base maps and graphs No. 101 HC, by permission of the University of Chicago Press.)

## TERMINOLOGY

To avoid cumbersome descriptions, the following terms, taken from various authors, are used in this paper, with the following meanings:

*Eggs*: deposited on the perennial or *primary* host.

*Fundatrices*: apterous, parthenogenetic viviparous females which hatch from the overwintered eggs and which normally give birth to:

*Fundatrigeniae*: apterous, parthenogenetic viviparous females borne by fundatrices or by earlier generations of fundatrigeniae on the primary host.

*Migrantes*: alate, parthenogenetic viviparous females borne by the fundatrigeniae, which fly to the *secondary*, or summer hosts, where they give birth to:

*Alienicolae*: apterous, parthenogenetic, viviparous females borne by migrantes or by earlier generations of alienicolae on the secondary hosts.

*Gynoparae*: alate, parthenogenetic, viviparous females borne by alienicolae on the secondary hosts which fly to the primary host and give birth to oviparous females.

*Males*: alate sexual males, born on the secondary hosts as brothers of

the gynoparae, which fly to the primary hosts, and copulate with the oviparous females.

*Oviparous females*: small, apterous, sexual females, borne on the primary hosts by the gynoparae. These mate with the sexual males, and lay eggs.

In certain other species of aphids, for example, *Myzus pseudosolani* Theobald, which exhibit facultative or obligatory polyphagy, the males are apterous, as are the oviparous females, and in this case the winged parthenogenetic females which fly to the primary hosts and give birth to these forms are called "sexuparae." Such a condition does not obtain in *Hyalopterus pruni*, however.

#### DESCRIPTION OF STAGES IN THE ANNUAL CYCLE

*Egg*.—The egg is regularly ovoidal and conforms to the usual type of aphid egg. It is black, but covered with a loose, sparse coating of silvery, waxlike rods, which cause it to appear gray to the unaided eye (fig. 2). When magnified about 15 times the egg appears black and sparsely covered with a shining white powder. The newly laid egg is light brilliant green, and is covered with white wax immediately after being laid. It darkens slowly and at the end of two or three days is entirely black.

Length, 0.47 to 0.66 mm; width, 0.22 to 0.35 mm; mean ratio  $W:L = 0.47$ .

*Fundatrix*.—First instar: head dark green with darker-green marking extending solidly across the vertex and posteriorly as two broad stripes across the occiput, leaving a narrow, lighter-green stripe in the mid-line and above each eye, not pulverulent; eyes dark reddish brown to black; antennae (fig. 3, A) very dark green to black. Thorax and abdomen uniformly dark green, dorsum not pulverulent, venter with a uniform coat of white meal; cornicles visible at moderate magnifications as round black dots, apparently not raised above the surface of the body. Antennae 4-jointed; length: 0.24 to 0.30 mm; mean, 0.26 mm. Length of the meta-tibiae, 0.13 to 0.18 mm; mean, 0.15 mm.

Second instar: same as first instar though coloring generally lighter; antennae 4-jointed; length: 0.32 to 0.40 mm; mean, 0.35 mm. Meta-tibiae length, 0.19 to 0.27 mm; mean, 0.22 mm (based on 60 specimens).

Third instar: same as adult instar, but considerably darker in color; markings and meal as in the adult. Antennae 5-jointed; length: 0.41 to 0.51 mm; mean, 0.46 mm. Meta-tibiae length, 0.28 to 0.40 mm; mean, 0.33 mm (based on 117 specimens).

Fourth instar: coloring and meal similar to adult, lighter in color than preceding instars. Antennae 5-jointed; length: 0.52 to 0.68 mm; mean, 0.61 mm. Meta-tibiae length, 0.42 to 0.56 mm; mean, 0.49 mm (based on 96 specimens).

Adult: apterous; body ovoidal (fig. 4); dorsum rounded or arched, not flattened as in succeeding generations; general color light green, of the same shade as the midrib of the young leaves of the plum, with three longitudinal darker green very irregular stripes, composed of minute irregular pigmented spots, one stripe mediadorsal and the others dorso-lateral; dorsum not pulverulent; venter covered with a thin, uniform coating of white meal. The mid-dorsal stripe is more distinct than the dorso-lateral stripes, venter concolorous, light greenish white, body segmentation indistinct. Head very pale green; antennae (fig. 3, B) colorless, translucent to pale

green, often black-tipped, occasionally with black or dark-gray color shading into the terminal half of the penultimate segment, borne on short frontal tubercles, length about one-third that of the body (for measurements see tables 1 and 2); eyes conspicuous, dark reddish brown to black; proboscis pale green, tip dark gray to black, reaching to meso-coxae. Legs vary from colorless, translucent, to yellowish light green; tibiae sometimes with apexes darkened; tarsi dark gray or black. Cornicles



Fig. 2.—Egg of *Hyalopterus pruni* (Geoff.) in its usual position at the base of the bud.

(fig. 3, L) dark brown to black, protruding very slightly above the body wall, conical; length, 0.053 mm; width, 0.049 mm. Cauda light yellowish green, or tipped with dark gray or black (fig. 3, Q), hairs on each side, 3 to 5 hairs in each row.

**Fundatrigenia.**—First instar: elongate, body straight, nearly parallel-sided, somewhat carinate, coloration and meal as in adult. Antennae (fig. 3, C) 5-jointed; length: 0.40 to 0.54 mm; mean, 0.48 mm. Tibiae, length: 0.24 to 0.35 mm; mean, 0.31 mm (measurements based on 82 individuals of the first generation).

Second instar: similar to the first. Antennae 5-jointed; length: 0.59 to 0.75 mm; mean, 0.68 mm. Tibiae, length: 0.40 to 0.48 mm; mean, 0.44 mm (based on 59 individuals of the first generation).

Third instar: body more rounded and tapering anteriorly than preceding instars;

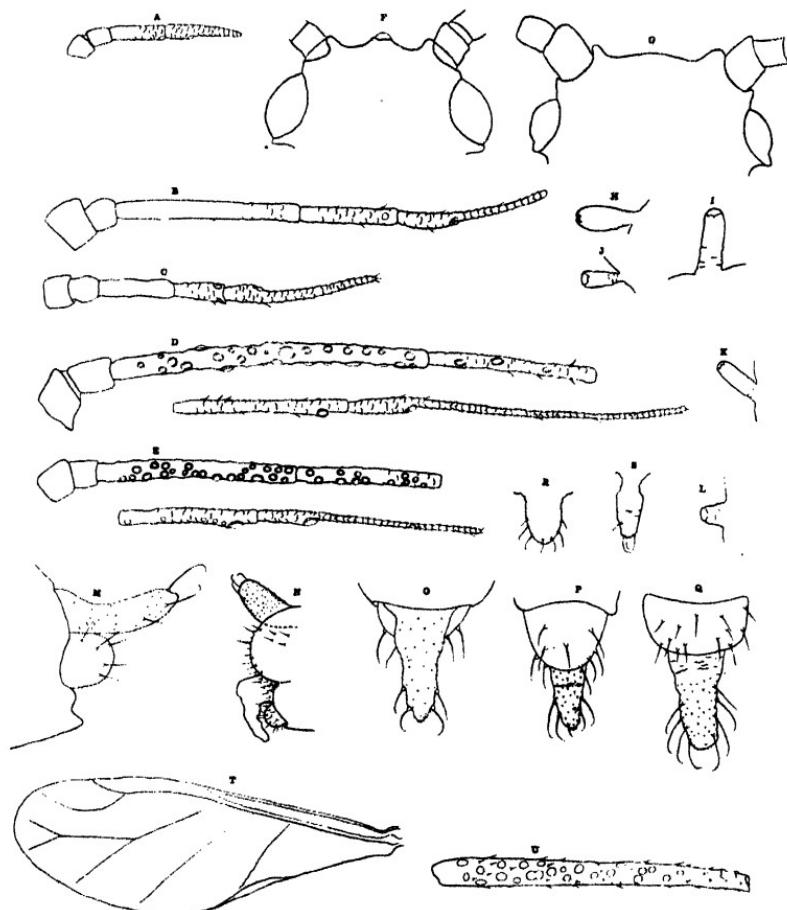


Fig. 3.—Anatomical taxonomic features of *Hyalopterus pruni* (Geoff.):

- A, Antenna, first-instar fundatrix.
- B, Antenna, adult fundatrix.
- C, Antenna, first-instar fundatrigenia.
- D, Antenna, adult migrans.
- E, Antenna, adult male.
- F, Dorsal view of head, migrans.
- G, Dorsal view of head, adult fundatrigenia.
- H, Cornicle, migrans.
- I, Cornicle, fundatrigenia.
- J, Cornicle, male.
- K, Cornicle, oviparous female.
- L, Cornicle, fundatrix.
- M, Cauda and anal plate, migrans, lateral view.
- N, Cauda and anal plate, male, side view.
- O, Cauda and anal plate, migrans, dorsal view.
- P, Cauda and anal plate, migrans, ventral view.
- Q, Cauda and anal plate, fundatrix, ventral view.
- R, Cauda, oviparous female, dorsal view.
- S, Cauda, male, dorsal view.
- T, Wing of gynopara.
- U, Meta-tibia, oviparous female.

Camera lucida drawings, all at the same magnification, except T.

coloration and meal as in the adult. Antennae 6-jointed; length: 0.82 to 0.99 mm; mean, 0.90 mm. Tibiae, length: 0.54 to 0.66 mm; mean, 0.60 mm (based on 84 individuals of the first generation).

Fourth instar: similar to adult. Antennae 6-jointed; length: 1.04 to 1.26 mm; mean, 1.16 mm. Meta-tibiae, length: 0.75 to 0.86 mm; mean, 0.81 mm (based on 50 individuals of the first generation).

Adult (fig. 5): body somewhat fusiform, rounded at the ends, with the greatest transverse diameter slightly posterior to the middle, dorsum convex, but not as highly arched as in the fundatrix; general color light green with three darker-green

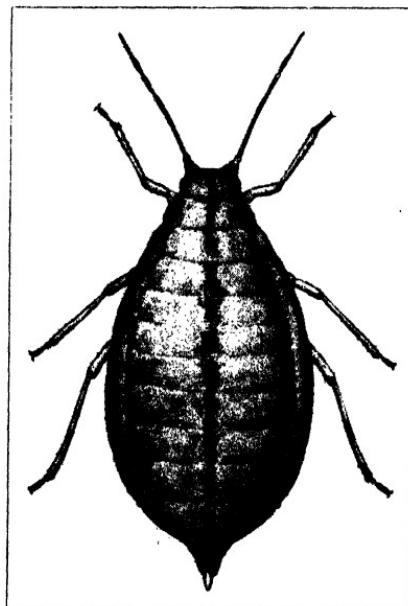


Fig. 4.—Fundatrix. The body is rounded and the antennae and legs are relatively short.

Figures 4 to 8 are at the same magnification.

stripes situated as in the fundatrix, but lighter in color; white meal arranged in four longitudinal bands on the dorsum of thorax and abdomen, each band composed of a circumscribed area on each body segment; venter of thorax and abdomen uniformly covered with white meal. Head light green, uniformly pulverulent; eyes dark reddish brown to black; antennae 6-segmented, about two-thirds as long as the body (for measurements see tables 1 and 2), borne on short frontal tubercles (fig. 3, G), translucent light yellowish green, with VI dark gray or black, V often dark in the distal half; proboscis hyaline, pale green, apex dark gray or black, extending to meso-coxae. Legs yellowish or greenish white, translucent, tips of tibiae and tarsi dark gray or black. Cornicles straight-sided or rarely somewhat fusiform (fig. 3, I), apex without flange, slightly imbricated; length, 0.116 mm; width, 0.040 mm; dark

gray or black. Cauda pale green or tipped with dark gray or black, a row of inward-curved hairs on each side, 2 to 3 hairs in each row.

*Migrans*.—First and second instars: identical to those of *fundatrigenia*, antennae 5-jointed.

Third instar: like the second, but with slight swellings on the thorax, the first indication of wing-pads, antennae 6-jointed.

Fourth instar (pupa): thorax twice as broad as apterous form; wing pads pale green, dark gray along the margins; otherwise color and pulverulence as in the adult.

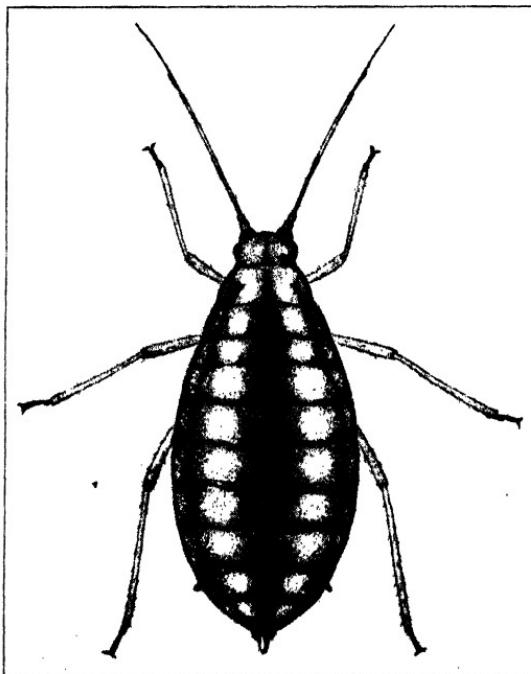


Fig. 5.—*Fundatrigenia*. The body is elongated and the antennae and legs are relatively long.

Antennae 6-jointed; length: 1.04 to 1.34 mm; mean, 1.19 mm. Tibiae, length: 0.51 to 0.85 mm; mean, 0.68 mm (based on 138 specimens).

Adult (fig. 6): head and thorax dark brownish or grayish black to black; abdomen light green; the whole body pulverulent, hairs sparse and small. Eyes very dark reddish brown to black. Antennae (fig. 3, D), about two-thirds as long as the body, borne on very short frontal tubercles (fig. 3, F), 6-jointed (see tables 1 and 2 for length of segments), very dark brown or black throughout, or with the base of III light brown or pale yellow; secondary sensoria circular, membrane convex, arranged in a row on IV; III with 18-30 sensoria; IV with 3-8; V with 0-2. Proboscis light yellowish green to dark green with tip light gray to black, extends to midway between the pro- and meso-coxae. Prothorax dark gray or black or with the posterior margin green; mesothorax, lobes very dark brown to black, pleural sclerites and venter dark

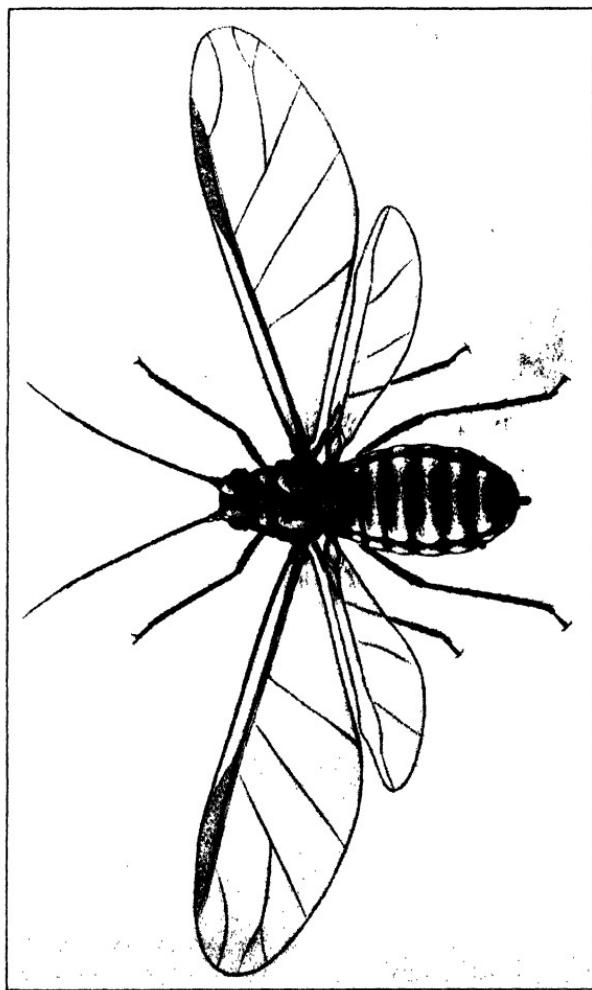


Fig. 6.—Migrans. Gynoparae are identical in appearance to this form.

brown to black, integument between pleural sclerites dark green, thorax uniformly pulverulent. Wings transparent, without markings, iridescent; veins light reddish brown except the heavy subcosta, which is light green; point of attachment of hamuli to fore wing, brown; stigma green with brownish borders; wing insertions light green; media twice-branched. Legs light green to nearly black, darker at the apexes of femora and tibiae, shading into green at the bases of these segments; tarsi black; coxae green to black. Abdomen with two dorsal rows of white mealy spots, one spot for each row on each segment of the abdomen, median darker-green line broadening toward base of abdomen, lateral darker lines indistinct and discontinuous; venter uniformly light green, pulverulent. Cauda (fig. 3, M, O, P), light green to dark gray or black, cylindrical, straight-sided, widening suddenly near the base, curved upward, bearing four or five hairs which are bent inwards near their apexes. Cornicles (fig. 3, H) black or with bases green, shining, glabrous, shorter than cauda; length, 0.113 mm; width 0.042 mm; club-shaped-cylindrical, not flared or flanged at the tip; slightly imbricated or smooth; held nearly perpendicular to the body, in life. Lateral papillae transparent, colorless or pale brown, hemispherical; one pair on the prothorax and each abdominal segment.

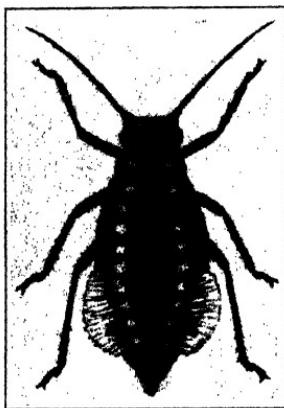


Fig. 7.—Oviparous female, showing lateral tufts of wax-like rods which serve to camouflage the egg.

*Oviparous Female*.—First instar: color and pulverulence as in the adult, with the exception of the lateral patches of white meal, which are lacking in the immature stages. Antennae 5-jointed; average length 0.36 mm. Average length of tibia 0.17 mm (based on 39 specimens).

Second instar: like the preceding; antenna, average length, 0.41 mm; tibia average length 0.21 mm.

Third instar: like the preceding; antenna, average length 0.52 mm; meta-tibia, average length 0.33 mm.

Fourth instar: like the preceding; antenna 5-jointed, average length 0.62 mm; average length of meta-tibia 0.39 mm.

Adult (fig. 7): apterous; general color light green to smoky yellow. Head light green, light yellow, or pale pink; eyes dark reddish brown to black; proboscis pale green, tip dark gray to black; antenna 6-jointed, often with III and IV fused, basal portion light green, distal portion of V and tip of IV dark gray to black. Thorax yellowish green, rarely pale pink, pulverulent pattern continuous with that of abdomen. Legs pale green with darker green or gray shading on the apexes of femora and tibia; tarsi black; color of meta-legs darker than pro-legs, meta-tibiae (fig. 3, U) slightly swollen throughout their length, bearing from 33 to 58 small circular sensoria. Abdomen with two large elliptical patches of white on each side, extending from about segment 4 to 8 inclusive, composed of silvery, reflective rods, sometimes

*Alienotola*.—Identical to the corresponding stages of the fundatrigenia.

*Gynopara*.—Identical in all stages to those of the inigrans, except in number of sensoria: III with 21–34 sensoria, IV with 6–12, V with 0–2. Wing shown in figure 3, T.

as long as the distance between the antennae, projecting postero-laterally as conspicuous tufts; a darker-green stripe extends along the mid-dorsal line of the thorax and abdomen, widening in the middle of the body; on either side of this a pulverulent line, simulating a light-gray stripe; on either side of these gray stripes a somewhat darker-green lateral stripe; venter uniformly pale yellowish green, lateral mealy patches conspicuous, extending one-third of the distance to the mid-ventral line

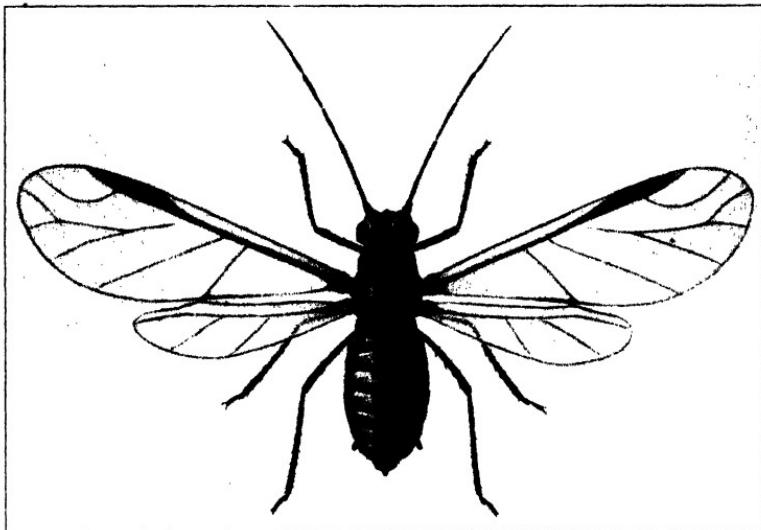


Fig. 8.—Male.

(and about as far onto the dorsum). Cornicles (fig. 3, K) black or dark brown, sometimes with base green; often partially imbedded in lateral mealy tufts; slightly or not at all imbricated; length, 0.067 mm; width, 0.028 mm; held perpendicular to body and diverging from each other at an angle of 30°. Cauda and sometimes last abdominal segment light green to dark gray or black, cauda (fig. 3, R) usually dark.

**Male.**—Fourth instar (pupa): coincident with pupae of gynoparae but easily distinguished by general body color. Body pale yellowish brown, rarely pale green, pulverulence as in fourth-instar gynoparae; eyes dark brown to black; antennae, segments I and II pale brown, III-VI inclusive dark brown to black; proboscis yellow, tip black; legs hyaline, tarsi dark gray; wing-pads brown with dark brown around the margins; venter of abdomen with pulverulence delimited by segments.

**Adult (fig. 8):** alate; head and thorax dark brown to black; eyes reddish brown; proboscis light greenish yellow, tip dark brown. Antennae (fig. 3, E) dark brown, lighter toward the tip, secondary sensoria circular; III, 32 to 48 sensoria; mean, 39.7; IV, 13 to 25 sensoria; mean, 19.8; V, 5 to 18; mean, 10.3 (for measurements of antennal segments see table 1). Prothorax dark brown with anterior and posterior margins dark green, lobes dark brown to black, integument between sclerites of pleurae yellow; venter of thorax dark brown; head and thorax glabrous to very slightly pulverulent. Abdomen, basic color yellow with a green spot on each segment simulating a discontinuous median and two lateral lines; dorsum glabrous; venter

thinly powdered. Cornicles (fig. 3, *J*) somewhat claviform, short; length, 0.071 mm.; width, 0.028 mm.; dark brown. Cauda, anal plate, and accessory lobes (fig. 3, *N, S*), dark brown to black.

TABLE 1

LENGTHS IN MILLIMETERS OF ANTENNAL SEGMENTS OF VARIOUS ADULT FORMS  
IN THE YEARLY CYCLE

Form	Antennal segment						Author	
	I	II	III	IV	V	VI		
Fundatrices	0.075 0.063	0.060 0.054	0.30 0.30	0.14 0.15	0.22 0.22	none none	0.80 0.79	Davidson ( <sup>16</sup> ) Present writer
Fundatrigeniae	0.085 ..... 0.073	0.075 0.45 0.067	0.39 0.45 0.43	0.24 0.30 0.27	0.22 0.25 0.22	0.44 0.57 0.48	1.45 1.77 1.54	Davidson ( <sup>16</sup> ) Lowe ( <sup>20</sup> ) Present writer
Migrantes	0.090 ..... 0.080 0.071	0.060 0.41 0.40 0.061	0.39 0.41 0.40 0.33	0.26 0.27 0.25 0.24	0.23 0.23 0.20 0.21	0.48 0.53 0.50 0.49	1.51 1.60 1.49 1.40	Davidson ( <sup>16</sup> ) Gillette and Palmer ( <sup>27</sup> ) Oestlund ( <sup>44</sup> ) Davidson ( <sup>16</sup> ) Present writer
Alienicolae	0.080 0.073	0.045 0.064	0.26 0.42	0.16 0.29	0.15 0.22	0.40 0.52	2.00 1.10	Gillette and Palmer ( <sup>27</sup> ) Davidson ( <sup>16</sup> ) Van der Goot ( <sup>29</sup> ) Present writer
Gynoparae	0.070 0.073	0.060 0.064	0.39 0.42	0.23 0.29	0.17 0.22	0.48 0.52	1.40 1.50	Davidson ( <sup>16</sup> ) Present writer
Males	0.080 0.064	0.070 0.058	0.38 0.35	0.25 0.23	0.23 0.22	0.46 0.46	1.47 1.38	Davidson ( <sup>16</sup> ) Present writer
Oviparous females	..... 0.060 0.051	..... 0.035 0.045	0.05 0.14 0.17	0.23 0.06 0.10	0.05 0.08 0.12	0.22 0.24 0.30	0.72 0.62 0.79	Lowe ( <sup>20</sup> ) Davidson ( <sup>16</sup> ) Present writer

\* Van der Goot gave ratios, so 0.480 was arbitrarily selected as a basis.

TABLE 2

## RATIOS OF THE LENGTHS OF ANTENNAL SEGMENTS

Form	Antennal segment					
	I	II	III	IV	V	VI
Fundatrices	1.2	1.0	5.3	2.6	3.9	...
Fundatrigeniae	1.1	1.0	5.9	3.8	3.3	7.0
Migrantes	1.3	1.0	6.5	4.3	3.6	8.3
Alienicolae	1.4	1.0	7.1	4.8	4.0	8.5
Gynoparae	1.2	1.0	6.0	4.2	3.2	8.2
Males	1.1	1.0	5.7	3.8	3.5	7.2
Oviparous females*	1.4	1.0	3.9	2.0	2.6	6.9

\* Exclusive of the measurements by Lowe, (<sup>20</sup>) which are obviously incorrect.

Several authors have recorded the length of the segments of the antennae of the various stages, either in taxonomic or biological observations on this species. These figures have been converted into millimeters, and are presented in table 1, together with measurements made by the present writer.

The figures presented in table 1 indicate a great variation in the length of the various segments of the antennae of the several adult forms of the mealy plum aphid. These differences are the result of variation in size of the aphids in response to environmental factors, and to differences in technique of preparing and measuring specimens. In view of the variability of the measurements, ratios seem to be more dependable. In table 2 are presented ratios based on a summation of table 1.

#### LIFE HISTORIES AND YEARLY CYCLE IN CALIFORNIA

The eggs (fig. 2), deposited in the late fall, are usually placed in the axils of lateral buds. When three or four buds occur in a cluster, as on the tip of a fruit spur, the eggs are usually wedged into the cavity enclosed by the bases of these buds. Such eggs are entirely hidden from view. Small cracks or crevasses in the bark are also utilized for oviposition. Eggs are never found exposed on the smooth bark of year-old wood. As a result of their position, the eggs are submerged in water during a considerable part of the winter. Water from rain, dew, and fog collects on the twigs and runs down to the lateral buds where the force of surface tension causes it to be retained between the bud and the twig. The eggs are often submerged, in this manner, to a depth of 1 or 2 millimeters. This fact bears an important relation to commercial spraying for control.

During the spring of 1932, the rate of hatching of the eggs was recorded. The eggs on twigs were kept in a lath-house at San Jose. This condition was presumed to simulate the alternate light and shade produced by the higher limbs of a tree upon the lower. Each day during the hatching period the twigs were shaken over a large sheet of white paper. A few moments after shaking, all dirt and débris could be blown from the paper without dislodging any of the newly hatched aphids. They were then easily counted, for they contrasted with the white background. These data are presented in table 3. A thermograph in a standard Weather Bureau kiosk, about 50 yards away, supplied the temperature records.

Although the eggs are usually laid during two months or more in the fall, they all hatch in about ten days in the spring. This indicates that the elapsed time from deposition to hatching is not constant. The cumulative percentage hatched is presented graphically in figure 9, together

with the temperatures for this period and for the preceding three weeks. This graph suggests that when the mean daily temperature approached 14° C., hatching was initiated. Roughly 6 to 10 days later 50 per cent of the eggs had hatched.

The various species and varieties of *Prunus* which serve as primary hosts for the mealy plum aphid, are in various stages of development during the hatching period. In California, the common hosts, prunes and European plums, generally have the white petals visible beyond the

TABLE 3  
HATCHING OF EGGS AT SAN JOSE, 1932

Date, March, 1932	Mean tempera- ture, °C	Number hatched	Per cent hatched	Summation of per cent hatched	Date, March, 1932	Mean tempera- ture, °C	Number hatched	Per cent hatched	Summation of per cent hatched
6	8.9	52	10.6	10.6	14	13.5	0	0.0	90.5
7	6.5	108	22.0	32.6	15	11.5	6	1.2	91.7
8	7.8	16	3.3	35.9	16	13.4	17	3.5	95.2
9	7.9	38	7.8	43.7	17	11.4	16	3.3	98.5
10	7.0	36	7.3	51.0	18	13.0	6	1.2	99.7
11	7.5	64	13.1	64.1	19	14.5	1	0.2	99.9
12	9.2	65	13.3	77.4	20	14.1	1	0.2	100.1
13	9.9	64	13.1	90.5					

green sepals by the time hatching is well started. This is commonly known as the "popcorn stage," or the "white-bud stage." By the time 5 or 10 per cent of the flowers have reached full bloom, approximately 90 to 95 per cent of the eggs have hatched. The survival of this species depends upon delaying hatching until the buds have opened, so that suitable feeding places will be available; but hatching cannot be delayed too long since a large percentage of the eggs are glued to the scales of the dormant buds, and they would fall from the trees with these scales, as the latter loosen and are blown away.

A few young fundatrices may emerge from the egg at the time the dormant fruit buds are just beginning to swell. As the buds elongate, the white bases of the scales become visible. The early aphids settle on this white tissue and apparently feed there. The majority of the newly hatched fundatrices, however, make their appearance about the time that the green sepals of the flower buds appear. They establish themselves on the sepals, usually with head towards the peduncle. In the Agen (French) prune, Grand Duke plum, and other species in which two or more flower buds arise from a single dormant bud, the aphids force their way between the appressed flower buds, and into the cup formed by the loosened dormant bud scales, wherein they establish themselves on the peduncles of the future flowers.

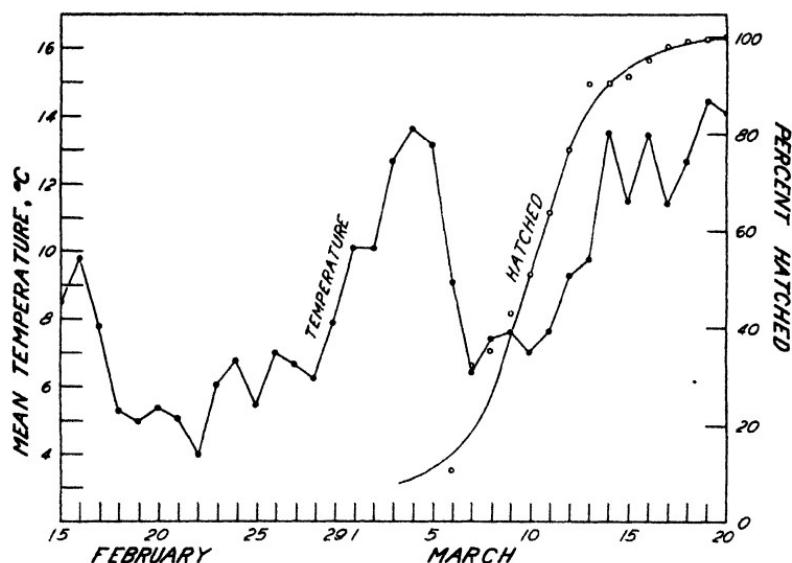


Fig. 9.—Hatching of eggs in the field, and concurrent temperatures, San Jose, 1932.

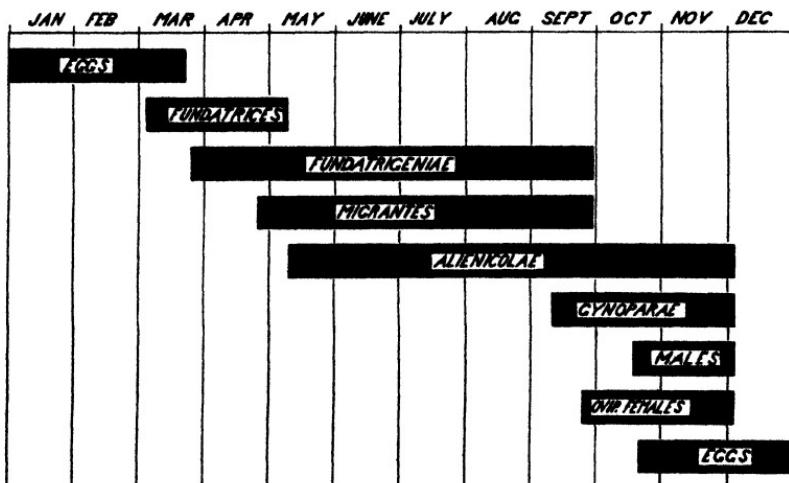


Fig. 10.—Seasonal occurrence of the various stages in the annual cycle, in California. (Data from table 4.)

At the time of full bloom, all of the eggs have hatched, and many second- and third-instar fundatrices can be found. In full bloom, the sepals of the flowers curl backwards, often forming a complete circle as they touch the calyx cup. The majority of aphids then take their positions on the inside of the recurved sepals.

When the calyx cup, or "jacket" as this portion of the perigynous flower of *Prunus* is commonly called, begins to dry and lose its succulence, the aphids move onto the lower surface of the young leaves. At about this time some of the fundatrices mature and give birth, parthenogenetically, to the first fundatrigeniae.

The dates of earliest and latest seasonal observations of the various stages, in several localities and on various hosts, are recorded in table 4. These data are represented graphically in figure 10.

The newly born fundatrigeniae settle in close proximity to the fundatrix, always on the lower surface of the developing leaves. When mature, they give birth parthenogenetically to another identical generation of fundatrigeniae and this is repeated until from three to ten generations of fundatrigeniae have been completed.

These aphids always attempt to establish themselves on the lower surfaces of the leaves, and are found on the upper surfaces or on petioles or twigs only under very unusual conditions. Gregariousness is a conspicuous trait of the fundatrigeniae and, in fact, of other stages of this species. The fundatrigeniae congregate in dense clusters on certain leaves, while other leaves in the immediate vicinity may be completely uninfested. This habit of gregariousness endows the aphids with the ability to curl the leaves of the plum. An early spring colony, consisting of a fundatrix surrounded by thirty or forty fundatrigeniae is capable of curling a young leaf. The curling does not partake of any of the characteristics of structures produced by typically gallicolous aphids. It consists of a simple rolling of the margins of the leaf toward the midrib, together with an arching or high convexity of the dorsal surface of the leaf, as seen in lateral view.

The fundatrigeniae increase in numbers in the Sacramento and San Joaquin valleys until the approach of very warm weather in the latter part of June or early part of July. At this time they occur in maximum numbers, and subsequently decrease rapidly. At the time of maximum infestation, plum orchards with a normally heavy infestation present a striking appearance. The mealy plum aphid produces copious sticky excrement, or honeydew, which coats the upper surfaces of the leaves below the colony and resembles varnish. At times, a black smut fungus grows in the excrement and causes the trees to appear blackened as

TABLE 4  
EARLIEST AND LATEST SEASONAL OCCURRENCE OF THE VARIOUS STAGES  
IN THE ANNUAL CYCLE

Form	Occurrence	Date	Host	Locality	Abundance
Eggs.	Earliest	{ 10/29/28 10/18/29 11/ 3/30 10/21/32	Plum	Davis Penryn Linden Linden	Rare Rare Common Rare
	Latest	{ 3/ 8/29 3/13/31 3/20/32	Plum	Davis Penryn San Jose	Rare Rare Absent
Fundatrices	Earliest	{ 3/ 5/29 3/11/29	Plum	Penryn Newcastle	Rare Common
	Latest	5/10/32	Plum	San Jose	Absent
Fundatrigeniae	Earliest	3/20/29	Plum	Penryn	Rare
	Latest	{ 9/ 1/28 9/ 1/30 9/30/30	Plum	Penryn Penryn San Jose	Absent Absent Rare
Migrantes	Earliest	{ 5/ 6/29 5/ 7/29 4/21/31 4/22/31 5/ 2/31 5/21/29 5/30/29 5/ 6/31 5/16/32	Plum	Davis Penryn Penryn Linden San Jose Penryn Reed Penryn Penryn	Rare Rare Rare Common Common Rare Common Common Common
	Latest	{ 7/13/29 8/13/29 9/ 1/30 9/ 4/28 10/ 3/29 9/ 1/30 9/29/31	Plum	San Jose Penryn Penryn Cattail Cattail Cattail Reed	Rare Rare Absent Absent Rare Rare Rare
Alienicolae	Earliest	{ 5/13/29 5/16/32	Cattail	Penryn	Common
	Latest	{ 12/ 3/29 11/17/32	Cattail	Penryn	Common
Gynoparae	Earliest	{ 10/ 5/28 10/ 2/29 10/ 6/30 9/10/31 9/ 8/32 10/16/28 10/22/31 9/20/32	Cattail	Penryn	Common
	Latest	{ 12/ 3/29 11/17/32 11/17/32	Cattail	Penryn	Rare
			Reed	Stockton	Common

\* Plum is used in this table to include "prune."

TABLE 4—(Concluded)

Form	Occurrence	Date	Host	Locality	Abundance
Males.....	Earliest.....	{ 11/ 8/28 10/18/29 10/31/32 10/28/32	Cattail Cattail Plum Linden Cattail	Penryn Penryn Penryn Penryn	Common Rare Rare Common
	Latest.....	{ 12/ 3/29 11/17/32 11/17/32	Cattail Reed Cattail	Penryn Stockton Penryn	Rare Common Common
Oviparous females.....	Earliest.....	{ 10/16/28 10/29/28 10/18/29 10/25/29 9/26/30 11/ 3/30 10/22/31	Plum Plum Plum Plum Plum Plum Plum	Penryn Davis Penryn Davis San Jose Linden Penryn	Common Rare Rare Common Rare Common Rare
	Latest.....	12/ 3/32	Plum	San Jose	Rare

though covered with soot. In heavy infestations the white molted skins stick in the excrement until the trees may appear light gray.

With the approach of warm summer weather, the fundatrigeniae give birth to young which develop into migrantes. As the summer advances the fundatrigeniae abandon the older leaves and congregate on the new, succulent growth at the tips of the branches. The young migrantes do not move in this manner, so that they are frequently found almost unmixed with fundatrigeniae on the older, lower leaves.

The fundatrigeniae are unable to endure the high midsummer temperatures of the Sacramento and San Joaquin valleys. They escape destruction for a time by releasing their hold on the lower surfaces of the leaves and allowing their bodies to hang vertically downward, suspended by the proboscis and one or both pro-tarsi. On hot days all of the aphids assume this position and remain thus throughout the middle of the day. But even this expediency proves insufficient in the interior valleys, and all of the fundatrigeniae are killed by the high temperatures. After the first period of unusually warm weather, myriads of dead fundatrigeniae can be found suspended to the lower surfaces of the exposed leaves, while a few survivors may be found in the coolest, shady portion of the tree. These few stragglers are killed by predators, or recurrent warm weather, so that orchards in the Sacramento and San Joaquin valleys are normally quite free from aphids by the middle of August.

In the Santa Clara and Sonoma valleys a few of the fundatrigeniae are able to withstand the hottest days of summer. In these cooler coastal

valleys, either a large percentage of the aphids are killed by the heat, or the fundatrigeniae are influenced to produce exclusively migrantes. However, a few escape and continue the line of fundatrigeniae. During July these survivors are very rare, but with the advent of cool fall weather they give rise to colonies. These colonies may persist until the leaves drop from the trees. They have misled many observers and have initiated the idea that this species was able to live on the trees year after year without the intervention of an alternate host. While it is true that in the coastal valleys the aphids or their eggs may be found on the trees at any time of the year, it is not true that they can survive a year on the trees in the absence of an alternate host. In the Santa Clara Valley the writer has frequently seen colonies of fundatrigeniae which persisted on the trees in considerable numbers until the arrival of gynoparae from secondary hosts. The gynoparae soon gave rise to oviparous females, so that the colonies often contained fundatrigeniae, migrantes, gynoparae, oviparous females, and males.

In northern California, the egg alone possesses ability to endure the winter. The ability to produce eggs is restricted to the oviparous females; and the ability to bear oviparous females is restricted to gynoparae. The spring forms on the primary hosts, that is, the fundatrigeniae and migrantes, cannot give rise to forms capable of depositing eggs. The writer has closely observed field colonies of fundatrigeniae on experimental trees in a lath-house at San Jose during the fall of 1930 and of 1932. They persisted until all the leaves fell from the trees in the early winter, but at no time were other forms than fundatrigeniae and migrantes produced.

Migrantes are produced by the fundatrigeniae over a long period of spring and summer, as indicated in table 4. They occur in maximum numbers on the trees during the latter part of June and early part of July. As the fourth-instar nymphs, or "pupae" molt to the adult stadium, they shed their pulverulent patches with the exuvium and appear glabrous.

Blakey<sup>6</sup> misinterpreted this phenomenon as follows: "There is a certain proportion of the winged forms about the third generation that divest themselves of their mealy covering, no doubt in preparation for migration." On the contrary, newly molted, glabrous specimens do not leave the primary host but remain there for several hours or even one or two days, until fully hardened and a conspicuous pulverulence has appeared.

The presence of migrantes on the trees has led to the popular misconception that these alate forms are responsible for the spread of this

species from tree to tree and from orchard to orchard. A number of publications record this idea as a fact. Banks<sup>(1)</sup> states "Winged specimens are occasionally developed which migrate to other trees." Blakey<sup>(2)</sup> states: "In August winged females are produced [which] only travel from place to place on the same tree." Theobald<sup>(3)</sup> states: Migrantes "fly from tree to tree." Theobald,<sup>(4)</sup> referring to migrantes, states: "These alatae fly away, some to settle on other plums. . ." Willcocks<sup>(5)</sup> states that sum-

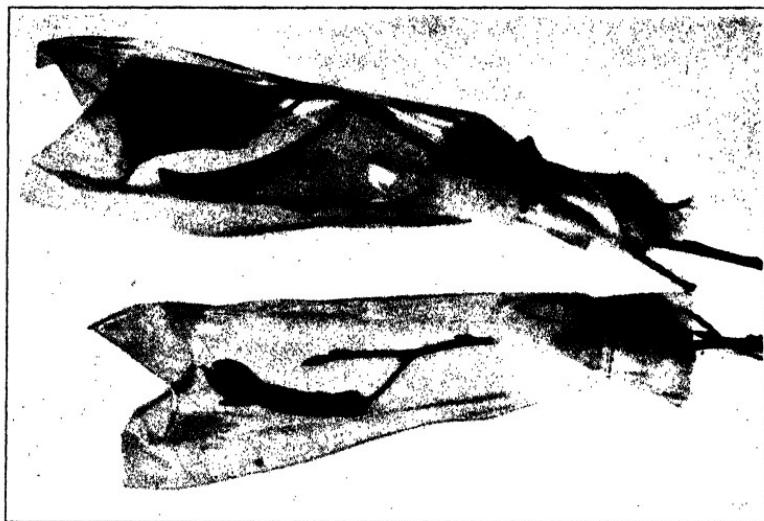


Fig. 11.—Cellophane bags used to cage aphids on trees in the field.

mer migrantes "fly off to other apricot or peach trees. . ." In addition, all taxonomists who saw fit to describe the aphids from the primary hosts as a distinct species, denied the fact of alternation of host plants, and implied that migrantes moved only to other primary hosts.

The question of the spread of this species in the orchards by the medium of the migrantes is of primary importance to fruit growers concerned. In order to shed additional light on this point, the writer during the seasons of 1929-1932 repeatedly caged migrantes on plum and prune trees. Transparent paper bags, described by Smith<sup>(6)</sup> and shown in figure 11, and cloth cages out of doors and in a lath-house were used. Both types of cages were shown to be successful when used to confine any particular form on its proper host. Migrantes when confined to plum or prune, failed to settle on the leaves, but walked or flew about the cage. At the expiration of 3 to 5 days, all migrantes became moribund, shrunken as though from starvation, and soon died. In no case were

young borne by migrantes on plum or prune. Apparently some peculiar change occurs in the migrans at the time of the fourth molt, which prevents the adult from obtaining nourishment from the plant which constituted the pabulum of the nymphal instars. Consequently, removal to secondary hosts is obligatory in the case of the migrantes, not facultative, as recorded by the above observers; and this alate form *does not* serve to distribute the species from tree to tree or directly from orchard to orchard.

Migrantes do not fly during the warmest part of the day, nor at times of considerable air movement. Their main flight period occurs from 4 to 7 in the evening, on clear, warm, still days. At such times in the latter part of June or early part of July in heavily infested areas the "air becomes full" of flying aphids. While driving 7 miles through a plum-raising section on such an occasion an average of 743 migrantes per square foot struck the windshield of the car.

On several occasions a flight of large numbers of migrantes was observed in the morning. Such a flight was observed at 10 o'clock in the morning on June 14 and 15, 1932, near San Jose. Hundreds of migrantes could be seen in the air, in all directions. The sky was completely overcast on both days, and there was little air drift. The temperature was 60° F on both mornings and the relative humidity 56 and 66 per cent, respectively.

The flight of the aphids is very weak, yet field evidence indicates that migration normally occurs over a distance of some miles. In 1928, all of the secondary hosts were removed for a radius of 4 miles around Davis. The following year the plums there suffered from an unusually heavy infestation of the mealy plum aphid. This indicates that migratory forms easily complete a flight greater than 4 miles.

Large stands of common reed, *Phragmites communis*, occur along the banks of drainage canals in the delta west of Stockton. Multitudes of migrantes arrive on these plants each year, although the nearest plum trees are 20 to 25 miles distant. The migratory tendency is strong in this species, as is shown by the fact that secondary host plants growing in heavily infested orchards, are frequently found to be less infested than hosts several miles distant from a source of migrantes. According to field evidence, this species normally migrates from 10 to 30 miles, and considerably greater distances may be possible. Under favorable conditions of climate and host plants, the mealy plum aphid would extend its range, naturally, at the rate of 60 miles a year: 30 miles would be covered by the migrantes and another 30 miles by the gynoparae and males.

After arriving on the secondary hosts, the migrantes do not imme-

TABLE 5  
MODIFICATION OF FALL SEX RATIO ON SECONDARY HOSTS

Colony No.	Locality	Host	Date	Gynoparae		Males	
				Number	Per cent	Number	Per cent
1	Penryn	Cattail.....	10/ 5/28	54	100	0	0
2	Penryn	Cattail.....	10/ 5/28	15	100	0	0
3	Penryn	Cattail.....	{11/16/29 11/22/29}	7 5	20 10	28 43	82 90
4	Penryn	Cattail.....	{10/ 7/30 10/29/30}	28 47	93 55	2 39	7 45
5	Stockton	Reed.....	{10/ 9/30 10/29/30}	7 24	100 63	0 14	0 37
			{10/27/32 10/28/32}	5 3	71 75	2 1	29 25
6	Penryn	Cattail.....	{10/30/32 11/ 4/32 11/ 7/32 11/12/32}	4 10 29 0	44 16 74 0	5 54 10 7	56 84 28 100
			{11/ 1/32}	202	60	133	40
7	Penryn	Cattail.....	{11/ 4/32 11/ 6/32 11/11/32 11/14/32 11/17/32}	149 33 3 13 2	89 45 12 32 5	104 41 23 28 37	41 55 88 68 95
			{11/ 1/32 11/ 4/32 11/ 6/32 11/11/32 11/14/32 11/17/32}	35 33 8 3 3 7	25 15 13 6 6 12	105 186 55 49 47 52	75 88 87 94 94 88
8	Penryn	Cattail.....	{10/21/32 10/30/32}	160 55	86 70	25 24	14 30
9	Stockton	Reed.....	{11/ 1/32 11/ 4/32 11/ 6/32}	246 556 25	78 76 26	68 175 73	22 24 74
10	Stockton	Reed.....	{11/ 1/32 11/ 4/32 11/ 6/32}	61 272 48	85 76 48	11 87 53	15 24 52
11	Stockton	Reed.....	{11/ 1/32 11/ 4/32 11/ 6/32}	62 285 31	78 78 60	17 89 21	22 24 40
12	Stockton	Reed.....	{11/ 8/32 11/11/32 11/14/32 11/17/32}	39 26 23 5	53 39 29 5	35 41 56 103	47 61 71 98
13	Stockton	Reed.....	{11/ 8/32 11/11/32 11/14/32 11/17/32}	29 40 19 20	40 51 40 20	44 38 28 82	60 60 60 36
14	Stockton	Reed.....	{11/ 8/32 11/11/32 11/14/32 11/17/32}	44 86 43 116	45 34 37 32	58 71 74 246	55 66 68 68
15	Stockton	Reed.....					

diately settle down, but rather walk about until they encounter other migrantes resting on the leaves, then settle in the immediate vicinity. As a result of this gregarious tendency, migrantes are generally found in compact colonies on the secondary hosts and appear to have been developed there. A typical colony on a cattail blade on June 11, 1929, extended for 15 inches (on one side of the leaf only) and was composed of 148 migrantes.

TABLE 6  
SUMMARY OF SEX RATIOS, 1932

Date, 1932	Penryn, on cattails		Stockton, on reeds	
	Total aphids	Per cent males	Total aphids	Per cent males
October 21			185	13.1
October 27	7	28.6		
October 28	4	25.0		
October 30	9	55.6	79	30.4
November 1	475	50.1	465	20.6
November 4	536	64.2	1,464	24.0
November 6	137	70.1	151	58.6
November 7	39	25.6		
November 8			244	54.1
November 11	78	92.3	252	59.5
November 14	91	82.4	243	65.0
November 17	98	90.8	572	75.3

When space permits, the migrantes, after becoming established on a secondary host, move their bodies around in a circle with the proboscis as center. As a result a circle of white powder is deposited on the leaf. The radius of this circle is roughly equal to the length of the body of the migrant. Curiously, this rotation of the body does not twist and break the inserted stylets of the proboscis.

On the first or second day of their existence on the secondary host, the migrantes begin the parthenogenetic viviparous production of alienicolae (plate 1, A). These are apterous parthenogenetic viviparous females identical in appearance to the fundatrigeniae. The first alienicolae give rise to other alienicolae and this cycle is repeated for about three to ten generations. On common cattail, *Typha latifolia*, the alienicolae are almost always found packed in dense colonies on the inner, or flat, surface of the leaf. They seek shade and are usually found on that portion of the blade which receives the maximum of shade during the day. They are never found crowded between the leaf base and the stem, as is the case with the majority of other aphids found on the cattail.

On *Phragmites communis* they prefer the lower surfaces of the leaves.

although large colonies are sometimes found on the dorsal surfaces of the leaves. On both of these hosts, but more particularly on the latter, the alienicolae at times produce a drying and killing of the leaf tissue.

Throughout the fall (see table 4 for dates) the alienicolae sporadically produce gynoparae, interspersed with other alienicolae. Shortly after the appearance of gynoparae in large numbers, a few alate males are found. These are apparently likewise produced by unmodified alieni-

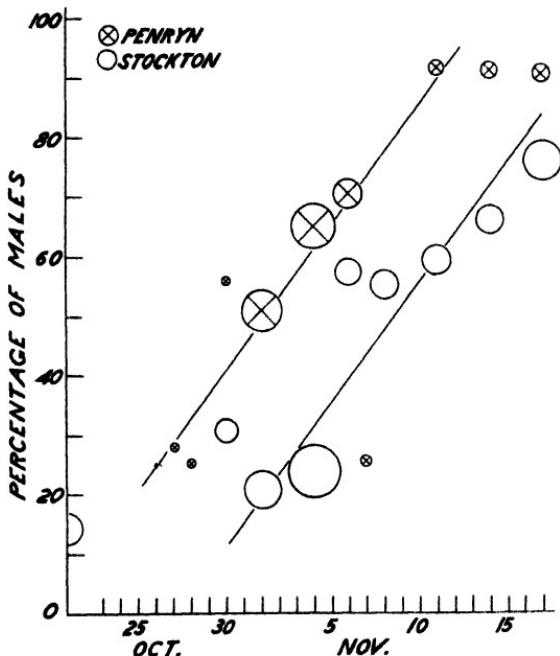


Fig. 12.—Trend in the sex ratio on secondary hosts. Areas of circles are proportionate to number of aphids in each count. Straight lines fitted by eye. (Data from table 6.)

colae. As winter approaches the number of males produced increases while the number of gynoparae produced decreases, as shown in table 5. The data presented in table 5 consist of counts made on specimens newly collected in the field or reared a few days later from material brought to the laboratory. Adults were identified as male or female by mounting on slides in euparial and examining genitalia and number of sensoria on antennal segment V. Later, counts were made by liberating living specimens on a window. The transmitted light accentuated their color differences, so that color, general size, and shape were used to classify them. These counts were checked periodically by microscopical examination.

For purposes of clarification and generalization the data obtained in 1932 have been summarized in table 6, and presented graphically in figure 12. The relative areas of the circles roughly represent the proportions of aphids used in determining each percentage. The straight lines representing the rate of increase of percentage of males on cattails at Penryn and on reeds at Stockton, have been fitted by eye. If the posi-

TABLE 7  
RATE OF PRODUCTION OF OVIPAROUS FEMALES BY GYNOPARAE

Gyno- para No.	Day after arrival on primary host										Total young	Total life of gynoparae on plum, days
	1	2	3	4	5	6	7	8	9	10		
Number of young born												
1	1	0	3	5	0	1	0	0	0	0	10	29
2	5	3	1	0	1	1	0	0	0	0	11	17
3	4	3	1	0	1	1	1	0	0	1	12	35
4	3	2	1	1	1	0	0	0	0	0	9	35
5	7	1	0	2	0	0	0	0	0	0	10	10
6	7	1	1	1	0	0	0	0	0	0	10	26
7	3	3	1	1	1	1	0	0	0	0	10	39
8	0	0	4	1	2	0	0	1	0	0	8	36
9	6	1	0	1	1	1	0	0	0	0	10	34
10	2	1	3	0	1	0	0	1	0	1	9	34
11	0	3	0	5	0	0	2	0	0	0	10	26
12	4	1	0	1	2	0	1	0	0	0	9	13
13	5	1	0	1	0	0	2	0	0	0	9	23
Totals	47	20	15	19	10	6	6	2	0	2	127	357
Averages	3.6	1.5	1.2	1.5	0.8	0.5	0.5	0.2	0.0	0.2	9.8	27.5

tion of the two straight lines is assumed to be correct, this graph indicates that the season at Penryn is about 7 days in advance of the season at Stockton.

The gynoparae are similar in most respects to the migrantes. The newly molted adults remain 1 or 2 days on the secondary hosts, then fly back to the plum and prune trees. They reach maximum numbers on the trees about the middle of November.

When gynoparae were confined on secondary hosts, they, like the migrantes confined on primary hosts, produced no young. However, when gynoparae were confined on plum or prune they immediately produced young, as shown in table 7.

As indicated in table 7, the gynoparae deposit their complement of young soon after arriving on the primary host, but may live for a considerable time thereafter. Two factors have no doubt fixed this habit of rapid deposition of the oviparous females. These are: firstly, the males

follow soon after the gynoparae, and often arrive on the trees before the oviparous females have matured; and secondly, normal leaf fall is usually initiated during the maturation of the oviparous females, and many of them fall to the ground on the abscised leaves.

The return flight of the gynoparae and males to the primary hosts is of importance to orchardists, since it is at this time only that the orchard receives an infestation. It has previously been shown that the migrantes do not directly distribute this species in the orchards. Certain phenomena, connected with the flight of the gynoparae, have led to much confusion in the interpretation of distribution and occurrence of this species. Numerous observers, particularly growers, have noted that heavy infestations occurred year after year on the same orchards, or on the same part of an orchard, while each year another portion of the orchard repeatedly remained free from aphid attack. As a matter of fact, this phenomenon is the rule, rather than the exception. It has led to the widespread conviction among growers that these aphids live the year round on the trees. The writer has had opportunity to observe on many occasions that trees which yearly showed a heavy infestation were in the immediate vicinity of a windbreak. The flight of the gynoparae is usually nondirective; it consists of a simple fluttering which does little more than maintain the aphid in mid-air. Horizontal movement is achieved by the aid of air currents. When the flying gynoparae enter the sheltered area near a windbreak they cease horizontal movement and after a period of fluttering, alight. Their repeated deposition on these trees is a process similar to "settling out" and is occasioned by the production of a body of stagnant air in the vicinity of a windbreak.

The gynoparae establish themselves on the lower surfaces of the leaves of the plum tree. They likewise manifest gregarious tendencies upon their arrival, but soon settle and generally remain fixed throughout the remainder of their lives.

The immature oviparous females are always found on the lower surfaces of the leaves, usually in close proximity to the gynopara which produced them (plate 1, *B*). At the fourth (last) molt, small circular sensoria-like organs appear on the meta-tibiae of the oviparous females. These structures are believed to serve the purpose of exuding an odorous material to attract the male. The males are certainly attracted to the females in some efficient manner, as is indicated by the following observation: In the fall of 1929, the mealy plum aphid occurred in greatly reduced numbers in Placer County. Only one gynopara, with her young could be found on about 1,000 leaves. Ten colonies were located after considerable search, and observed daily. They varied from 1 to 10 ovip-

arous females. Males may be assumed to have been no more numerous than the gynoparae which preceded them (table 6); yet each colony was visited by a male at least once.

Observations on the sexual cycle in cages, at Penryn in 1929 and at San Jose in 1932, have established the following points: (1) Oviparous females will not deposit eggs prior to copulation. In the absence of males these females remain stationary on the leaves. Such virgin females had lived for over 1 month, at which time death resulted from starvation since the leaves became detached from the twig. (2) A single copulation lasts from 5 to 30 minutes. (3) Males and females may both copulate repeatedly, and at intervals of 15 or more minutes. (4) A single copulation suffices to enable a female to deposit her full complement of eggs, although this process may require several days. (5) Oviposition usually occurs a few hours after copulation. (6) Males often combat for females. (7) Copulation and oviposition occur during the warmer part of the day. (8) Males which matured on secondary hosts at the same time as certain gynoparae, can maintain themselves on plum leaves until these gynoparae have given birth to young, and the young have reached maturity. Such old males successfully copulate and give rise to fertile eggs. Thus the changing sex ratio (fig. 12) is not absolutely essential to the survival of this species. (9) The maximum length of life of an oviparous female, from birth to death, was 49 days.

Oviposition is accompanied by a procedure rarely encountered in the Aphididae. The female backs into the axil of a bud, or occasionally into a depression in the bark. The pale green egg is then extruded slowly over a period of about 2 minutes. Then while standing over the egg, the female scrapes the waxlike rods from the pleurae and venter of the abdomen by means of the meta-tibiae, and applies them to the egg. This process is repeated over and over, slowly, for about 20 minutes and at the end of this period the egg has a uniform coat of white rods (fig. 2) which cause it to appear light gray to the unaided eye.

Several authors<sup>(22, 25, 26, 33)</sup> have described the egg as "black and shiny." When a female is removed artificially, immediately after depositing an egg and before the mealy covering has been applied, the egg finally appears black and shining. Such an interruption of the normal process rarely occurs in nature, and the author has not found black and shining eggs occurring naturally.

To gather further information on oviposition, seven cages were used, containing respectively 1, 2, 3, 3, 8, 9, and 37 oviparous females. These females were isolated from males for a few days after maturity, and no eggs were deposited. Males were introduced and egg deposition began a

few hours later. The eggs were counted daily with a hand lens, and the numbers of oviparous females surviving recorded. These data are summarized in table 8.

The total number of eggs is based on a count made with a binocular microscope at the end of the test. One isolated female laid 5 eggs, although the average was 3.3 eggs per female. This table indicates that a large percentage of the eggs are deposited within the first four days after mating.

TABLE 8  
RATE OF OVIPOSITION

Day after mating	Number of females	Number of eggs laid	Eggs per female	Mean temp., °C.
1.....	37*	22	0.59	11.7
2.....	60	35	.58	17.2
3.....	57	37	.65	18.6
4.....	49	31	.63	16.9
5.....	47	2	.04	15.0
6.....	45	12	.27	9.7
7.....	42	6	.14	9.7
8.....	38	11	.29	15.6
9.....	32	11	.34	12.2
10.....	31	3	.10	16.4
11.....	29	3	.10	17.2
12.....	27	1	0.04	17.2
Total.....	60	196†	3.3	

\* Data not recorded for the smaller cages on the first day.

† Microscopic count at end of test.

The average number of eggs which would be deposited on a tree following the arrival of a single gynopara may be computed from tables 7 and 8. This number would be the product of the average number of oviparous females produced by one gynopara, and the average number of eggs laid by one oviparous female, and amounts to 32.3 eggs. Field experience has shown that the arrival of a single gynopara (and one or more males) is sufficient to produce a severe infestation over the entire tree the following spring.

#### HOST PLANTS

In California the plum aphid is generally limited to three hosts, namely, plum, *Prunus domestica* Linn.; common reed, *Phragmites communis* Trin.; and cattail, *Typha latifolia* Linn. Of these, the two latter are secondary hosts.

In this state plums are usually grown for prune making. The total acreage planted to plums for table fruit and canning is much less than that planted to plums for drying. The four leading varieties used for

prunes, all of which belong to *Prunus domestica*, are Agen (French), Sugar, Sergeant (Robe de Sergeant), and Imperial. The writer has seen all of these heavily infested with *Hyalopterus pruni*.

In the Sierra foothills on the eastern side of the Sacramento Valley, many varieties of plums are grown for table fruit. In this area many blocks of Japanese plums, *Prunus salicina* Lindl. are grown contiguously with common garden plums, *Prunus domestica*. The varieties of

TABLE 9  
HOSTS ACCEPTABLE TO NEWLY HATCHED FUNDATRICES  
(Ten aphids per cage)

Cage	Date hatched, 1932	Trial host	Observations on aphids			
			March 17	March 21	March 27	April 11
1	March 6	Grand Duke plum.....	normal	normal	normal	about 200 aphids
3	March 12	Santa Rosa plum.....	normal	normal	normal	all dead
2	March 11	J. H. Hale peach.....	abnormal	all dead	.....	.....
6	March 12	Mayflower peach.....	abnormal	all dead	.....	.....
4	March 12	Mission fig.....	abnormal	all dead	.....	.....
5	March 12	Bing cherry.....	abnormal	abnormal	all dead	.....
7	March 13	Bose pear.....	normal	normal	normal	all dead
8	March 13	Comice pear.....	normal	normal	normal	all dead

*Prunus domestica* have nearly all been found to be infested by mealy plum aphid but not a single specimen of this species has been found on any of the varieties of *Prunus salicina*. It may be concluded that under California conditions, and probably elsewhere, Japanese varieties and most, if not all, Japanese hybrid varieties, are immune to plum-aphid attack.

Although *Hyalopterus pruni* has been recorded from many other species of *Prunus* in various parts of the world, there are but two reports of any other *Prunus* host in California. These reports<sup>(12, 22)</sup> pertained to slight infestations on apricot (*Prunus armeniaca* Linn.). The writer has at no time found *Hyalopterus pruni* occurring naturally on apricot. During the spring of 1931 large numbers of fundatrigeniae were transferred from plum to apricot and peach trees in cages like those shown in figure 11. No young were born on the peach and the aphids were all dead within a few days after transfer. Young were born on the apricot and a strong colony developed.

In order to avoid the possible complicating factor of individual adaptation of plum prior to transfer, or of adaptation of the parthenogenetic line, hosts were tested during the spring of 1932, using newly hatched fundatrices. The results are shown in table 9.

The results presented in table 9 are in harmony with field observations. The Santa Rosa plum is a variety of *Prunus salicina*, the Japanese plum, and is not a host under field conditions.

Immunity under field conditions might be the result of selection on the part of gynoparae. To test this hypothesis, ten gynoparae and ten males were confined on each of several hosts with the results indicated in table 10.

TABLE 10  
HOSTS ACCEPTABLE TO GYNOPARAE AND MALES  
(Ten each per cage)

Trial host	Number of oviparous females born at end of 9 days	Alates surviving after 9 days	Trial host	Number of oviparous females born at end of 9 days	Alates surviving after 9 days
Moorpark apricot.....	70	17	<i>Prunus americana</i> (wild).....	71	7
Moorpark apricot.....	0	20	Purple leaf myrobalan.....	48	6
<i>Prunus armeniaca</i> (wild).....	0	0	Bing cherry.....	0	0
Mayflower peach.....	30	4	Bartlett pear.....	0	0
J. H. Hale peach.....	0	0	Delicious apple.....	0	3
Grand Duke plum.....	72	19	Black fig.....	0	0
Wickson plum.....	4	15			

In this test several partial failures can be traced to the fact that the leaves abscised early and the aphids died of starvation. This was the case with Mayflower peach, J. H. Hale peach, *Prunus armeniaca* (wild) and the second cage on Moorpark apricot. The Wickson plum is a hybrid, probably *P. salicina* × *P. domestica*.

According to this test, the common garden plum, *Prunus domestica*; peach, *Prunus persica* Sieb. and Zucc.; and apricot, *Prunus armeniaca*, are capable of supporting gynoparae, males, and oviparous females. Field evidence indicates, however, that the alates do not seek the two latter species, under California conditions.

The favorite secondary host of *Hyalopterus pruni* is the common reed, *Phragmites communis*. This is a perennial graminaceous plant belonging to the tribe Festuceae. It is hydrophytic and grows equally well in fresh and brackish marshes. The erect culms generally attain a height of 8 or 10 feet, and carry alternate, lanceolate, flat leaves which are often 2 inches wide at the point of greatest width. Culms arise from submerged stolons which may attain a length of several feet. The aerial portions of this plant are killed by frost each winter, in northern California, and new culms arise each spring. Hence there is no opportunity for *Hyalopterus pruni* to survive the winter on this host. In the latter part of the summer the reed produces a large, loose, terminal panicle which persists

after fruition until after the death of the culm. The panicle becomes silvery white and generally droops slightly to one side. It serves as a ready indicator of this species, as it towers above all other marsh plants. The common reed is widely distributed in California and is particularly abundant along canal and river banks in the deltas of the Sacramento and San Joaquin rivers.

The only other secondary hosts in California, known to the writer, are the common cattail, *Typha latifolia*, and rarely *Typha angustifolia* Linn. The cattail (Typhaceae) is a hydrophytic plant found in shallow, lenitic and lotic fresh-water environments. The radical leaves, 3 to 6 feet tall, are usually flat on the inner surface and slightly convex on the outer. Internally they show a peculiar, porous structure. A few caudine leaves arise from the simple, nonsegmented culm. In this monoecious plant, the staminate flowers are borne in a loose spike immediately above the compact, smooth, velvety, brown cylindrical spike of pistillate flowers.

The aerial portions of this plant likewise are killed by winter frosts. The plants occur in dense colonies, and spread by means of stout, submerged rhizomes. The species is widely distributed in California.

*Typha angustifolia* is characterized by having the staminate portion of the spike removed an inch or two from the pistillate portion, whereas in *T. latifolia* these portions are contiguous. In Placer County these two species are sometimes found in mixed stands. In such cases, although *T. latifolia* is heavily infested, *T. angustifolia* may support but an occasional small colony.

In Placer County *Typha* is the only secondary host, since *Phragmites* does not occur there. In San Joaquin County, however, *Phragmites communis* and *Typha latifolia* frequently occur in mixed stands, and although *Phragmites* may be heavily infested with alienicolae, no colonies whatsoever have been found on *Typha*. It seems likely that the aphids in Placer County are adapted to cattail, and those in San Joaquin County to reed.

*Arundo donax* Linn., a host in other parts of the world, is not a host in California. Several stands of this plant have been examined in Placer, San Joaquin, and Santa Clara counties and no plum aphids found on them. Migrantes were placed on *Arundo* at San Jose, in cages, but bore no young, and soon died.

Large numbers of diverse plants have been recorded as hosts of the mealy plum aphid in the literature. Many of these plants have been subjected to cage tests at San Jose, and a considerable number have been found unsuited to this aphid. The list on pages 200-201 separates the known host plant species from those which are not hosts.

## I. Authenticated hosts of the mealy plum aphid:

NAME GIVEN IN LITERATURE	PRESENT ACCEPTED NAME
<i>Arundo</i>	
<i>Arundo donax</i>	<i>Arundo donax</i> Linn. (giant reed)
<i>Arundo phragmites</i>	
<i>Typha latifolia</i>	<i>Typha latifolia</i> Linn., common
Cattails	cattail
<i>Typha angustifolia</i> Linn.	<i>Typha angustifolia</i> Linn.
<i>Phragmites communis</i>	
Reed grass	
<i>Phragmites</i>	<i>Phragmites communis</i> Trin.,
<i>Phragmites phragmites</i>	common reed
<i>Phragmites vulgaria</i>	
<i>Phragmites arundo</i>	
<i>Phragmites kirki</i>	<i>Phragmites kurku</i>
<i>Phragmites kiski</i>	
Plum and prune	
<i>Prunus domestica</i>	
<i>Prunus</i> sp.	
European plum	
Pershore	
American plum	<i>Prunus domestica</i> Linn., com-
Greengages	mon garden plum
Violet Gage plum	
Gages	
Czar plum	
Victoria	
Monarch	
<i>Prunus insititia</i>	<i>P. domestica</i> var. <i>insititia</i> Bailey
Damson	(Damsons, Bullaces, Mirabelles, St. Juliens)
<i>Prunus spinosa</i>	
Sloe	<i>Prunus spinosa</i> Linn., black-
Blackthorn	thorn or European sloe
Peach	<i>Prunus persica</i> Sieb. & Zucc.,
<i>Prunus persicæ</i>	peach
<i>Prunus communis</i>	
Almond	<i>Prunus communis</i> Fritsch,
<i>Prunus amygdalis</i>	almond
<i>Amygdalus communis</i>	
Apricot	<i>Prunus armeniaca</i> Linn., com-
<i>Prunus armeniaca</i>	mon apricot
<i>Prunus americanus</i>	<i>Prunus americana</i> Marshall,
	American wild plum
Nectarine	<i>Prunus persica</i> var. <i>nucipersica</i>
	Schneid., nectarine

## II. Species formerly listed as hosts which are not hosts:

NAME GIVEN IN LITERATURE	PRESENT ACCEPTED NAME
<i>Calamagrostis arenarius</i>	
<i>Arundo epigeios</i>	
<i>Arundo erigyros</i>	
<i>Calamagrostis littorea</i>	
<i>Juncus</i> sp.	<i>Juncus</i> sp., rushes
Tules.	<i>Scirpus acutus</i> Muhl., common tule
<i>Scirpus lacustris</i>	<i>Scirpus lacustris</i> Vahl., great bulrush
<i>Scirpus caespitosus</i>	<i>Scirpus caespitosus</i> Linn.
<i>Amophila arundinacea</i>	<i>Anemophila arenaria</i> Link, beach grass
<i>Salix</i>	<i>Salix</i> sp., willow
<i>Salsola kali</i>	<i>Salsola kali</i> Linn., Russian thistle
<i>Umbellularia californica</i> Nutt.	<i>Umbellularia californica</i> Nutt., California laurel
Grape.	<i>Vitis</i> sp., grape
<i>Pyrus malus</i>	<i>Pyrus malus</i> Linn., apple
Apple	

## III. Doubtful hosts species:

NAME GIVEN IN LITERATURE	PRESENT ACCEPTED NAME
<i>Poa annua</i>	<i>Poa annua</i> Linn.,* common blue grass
<i>Elymus arenarius</i>	<i>Elymus arenarius</i> Linn.,* sea lyme-grass
<i>Phalaris arundinacea</i>	<i>Phalaris arundinacea</i> Linn.,* reed canary grass
<i>Dactylis glomerata</i>	<i>Dactylis glomerata</i> Linn.,* orchard grass
<i>Chenopodium album</i>	<i>Chenopodium album</i> Linn.,* white pigweed
<i>Phaseolus vulgaris</i>	<i>Phaseolus vulgaris</i> Linn.,* bean
<i>Buddleia madagascariensis</i>	<i>Buddleia madagascariensis</i> Lam.
<i>Prunus serotina</i>	<i>Prunus serotina</i> Ehrh., wild black cherry
<i>Prunus pissardi</i>	<i>Prunus cerasifera</i> var. <i>pissardii</i> Koehne
Choke cherry	<i>Prunus virginiana</i> Linn., choke cherry

\* Probably not a host.

In the above list a number of recorded hosts have been discredited for the following reasons:

*Calamagrostis epigeios*: Börner<sup>(9)</sup> after examining this plant repeatedly, failed to discover *Hyalopterus pruni* on it. From this and the nature of the plant, he concluded that it was not a host.

*Juncus sp.*: The only authority for this host is Walker.<sup>(6)</sup> His observations throughout indicate a lack of familiarity with *Hyalopterus pruni*. Since no other observer has recorded *H. pruni* on *Juncus*, this host may be rejected.

*Phragmites kirki* and *P. kiski*: These names were originated by van der Goot<sup>(29)</sup> and Theobald.<sup>(30)</sup> A. S. Hitchcock<sup>4</sup> writes: "There is no *Phragmites kirki* (nor *kiski*). It may be a misspelling for *P. karka*, a species of eastern Asia."

*Scirpus acutus*, *S. lacustris*, *S. caespitosus*: The writer has repeatedly examined several species of *Scirpus* in the field and at no time found *Hyalopterus pruni* on them. In addition attempts were made to colonize them on *Scirpus acutus*, in cages, without success.

*Ammophila arenaria*: The leaves of this species are hard and involuted to form a smooth rod. It occurs in exposed situations in shifting sand dunes. It bears no resemblance to the known hosts of the plum aphid. Cage tests at San Jose demonstrated that the aphid cannot survive on it.

*Salix*: This host was cited only by Walker<sup>(22)</sup> and has been discredited by Theobald.<sup>(31)</sup>

*Salsola kali*: This plant was recorded as a host by Walker,<sup>(22)</sup> and cited by Patch.<sup>(32)</sup> Walker's observations indicate that he was not dealing with the plum aphid, since he recorded a wingless male. Cage tests at San Jose indicated that the plum aphid cannot survive on *Salsola kali*.

*Umbellularia californica*: W. M. Davidson<sup>(16)</sup> collected migrants on this plant, during their normal movement to secondary hosts. They were transients.

*Vitis*: The plum aphid was cited on grape by Scopoli, according to Theobald.<sup>(33)</sup> Other investigators have sought the species in vain on this plant.

*Pyrus malus*: Tavares<sup>(34)</sup> is the only authority for the occurrence of the mealy plum aphid on apple. In view of the abundance of both apples and mealy plum aphids, more records would be expected if apple is actually a host. Cage tests at San Jose (see also table 8) indicated that the plum aphid cannot survive on apple.

From a study of the literature the following generalization can be drawn: (1) In the colder regions of the temperate zones *Hyalopterus pruni* attacks plums chiefly or exclusively, and *Phragmites communis* is the usual secondary host. (2) In the warmer temperate and tropical regions *H. pruni* attacks chiefly peach, apricot, and almond, while *Arundo donax* and *Phragmites communis* serve as secondary hosts. This peculiar host specificity, related to climate, is no doubt the result of the

<sup>4</sup> Personal communication, 1932.

importation of the normally plum-feeding aphid into the warmer regions where plums are rare, and finally into the tropics where plums are absent, with the result that the aphid became adapted to peach, apricot, and almond.

#### PARASITES AND PREDATORS

Although Gillette and Taylor<sup>(28)</sup> stated that the mealy plum aphid "seems to have few natural enemies . . ." the majority of observers are of the opinion that such enemies are numerous. The writer has seen the plum aphid reduced nearly to extinction by predators, in wide areas of California.

Fundatrices appeared in enormous numbers on the opening buds of plum in the Linden area, early in the spring of 1930. Their abundance predicted an abnormally heavy infestation later in the spring. However, at about the time the fundatrices were establishing colonies of fundatrigeniae, several species of *Podabrus* occurred in large numbers. They reduced the aphid population so effectively that many orchards were virtually free from attack that year.

At the peak of the occurrence of fundatrigeniae, they are found to be heavily attacked in all infested areas of California, chiefly by coccinellids, several species of *Podabrus*, and several species of syrphids. Hemerobiids, chrysopids, and the internal parasite *Praon simulans* (Prov.) are present, but not important factors in reducing the numbers of aphids. Dobrovliansky<sup>(29)</sup> reared a hyperparasite, *Lygocerus* sp., from *Praon flavinode* Hal. in Russia. This hyperparasite has not been reared by the writer in California. It may be present, however, and may account for the lack of efficiency of *Praon simulans* in reducing the plum aphid more effectively.

The same species which feed on the plum aphid in the orchards in California, likewise attack it on secondary hosts. On cattails in Placer County, syrphid larvae have predominated during the last five years. On reeds in San Joaquin County, coccinellids and larvae of *Leucopis* have been most abundant.

During the fall of 1932 at San Jose, an unidentified species of *Geocoris* occurred on trees infested with plum aphids. Both nymphs and adults of *Geocoris* frequently impale plum aphids on their probosces after the manner of *Orius*. But the struggles of the impaled aphids apparently frightened these pseudo-predators, so that they usually withdrew. Occasionally, however, an aphid was eaten.

The total number of species which feed on the mealy plum aphid is, no doubt, very great. No serious attempt has yet been made to discover

and identify them. A number of investigators have, however listed occasional parasites and predators which have come to their attention. These are grouped phylogenetically in table 11.

TABLE 11  
PARASITES AND PREDATORS WHICH FEED ON MEALY PLUM APHID

Order and family or species	Country	Authority*
<b>Neuroptera</b>		
Hemerobiidae, various species . . . . .	U.S.A.	(16)
<i>Chrysopa cognata</i> . . . . .	Japan	(45)
<i>Chrysopa californica</i> . . . . .	U.S.A.	(63)
<i>Chrysopa</i> sp. . . . .	Morocco	(38)
Chrysopidae, various species . . . . .	U.S.A.	(16)
<b>Hemiptera</b>		
<i>Triphleps (Orius) sp.</i> . . . . .	U.S.A.	(16)
<i>Geocoris</i> sp. . . . .	U.S.A.	(writer)
<b>Coccoptera</b>		
<i>Hippodamia convergens</i> Guerin . . . . .	U.S.A.	(16, 26)
<i>Coccinella septempunctata</i> . . . . .	Morocco	(38)
<i>Scymnus subtillosus</i> var. <i>pubescens</i> . . . . .	Morocco	(38)
<i>Scymnus suturalis</i> . . . . .	Morocco	(38)
<i>Donacia clavipes</i> . . . . .	England	(42)
<i>Cantharis fusca</i> L. . . . .	Russia	(36)
<i>Cantharis obscura</i> L. . . . .	Russia	(36)
<i>Podabrus comes</i> Le C. . . . .	U.S.A.	(16)
<i>Podabrus binotatus</i> Le C. . . . .	U.S.A.	(16)
<i>Podabrus pruinatus</i> Le C. . . . .	U.S.A.	(writer)
<i>Telephorus divisus</i> Le C. . . . .	U.S.A.	(16)
<b>Diptera</b>		
Syphidae, various species . . . . .	U.S.A. France Russia	(16) (48) (20)
<i>Catolomba pyrastris</i> Lin. . . . .	U.S.A.	(11, 16)
<i>Syrphus albomaculatus</i> . . . . .	Morocco	(38)
<i>Syrphus americanus</i> Wied. . . . .	U.S.A.	(11)
<i>Euphedges volucris</i> O. S. . . . .	U.S.A.	(26)
<i>Leucopis</i> sp. . . . .	U.S.A.	(16)
<i>Leucopis annulipes</i> Zett. . . . .	Germany	(7)
<i>Bremia</i> sp. . . . .	Russia	(20)
<i>Aphidoletes meridionalis</i> . . . . .	U.S.A.	(18)
<b>Hymenoptera</b>		
Hymenopteran, undetermined . . . . .	Morocco	(38)
<i>Praon flavinode</i> Hal. . . . .	Russia	(20)
<i>Praon simulans</i> (Prov.) . . . . .	U.S.A.	(writer)

\* Numbers in parentheses refer to "Literature Cited," at the end of the paper.

## SUMMARY

The proper name for the mealy plum aphid is *Hyalopterus pruni* (Geoff.).

The mealy plum aphid is widely distributed in the world: it is reported from 27 countries, and from 15 states in the United States.

The stages in the annual cycle are: egg, fundatrix, fundatrigenia, migrans, alienicola, gynopara, alate male, and oviparous female. Each instar of these forms is described.

Minute differences exist between the two alate stages, migrantes (named *Hyalopterus pruni* by Fabricius) and gynoparae (named *H. arundinis* by Fabricius).

The hatching period is about two weeks in length.

The seasonal occurrence of each form is given.

Migrantes cannot survive on plum, after the fourth molt, and do not serve to distribute the aphids from tree to tree. They can survive only on the secondary hosts. Distribution of this species in the orchards is achieved only in the fall during the return flight of the gynoparae. Each migration may cover 30 miles, which makes possible natural spread at the rate of 60 miles a year.

The percentage of males on the secondary hosts increases throughout the fall and approximates 100 per cent late in November.

Each gynopara produces an average of 9.8 oviparous females.

Oviparous females deposit an average of 3.3 eggs each.

The primary hosts of the mealy plum aphid in California consist only of varieties of *Prunus domestica* Linn. The secondary host in Placer County is the cattail, *Typha latifolia* Linn.; in San Joaquin County the secondary host is the common reed, *Phragmites communis* Trin.

Many hosts cited in the literature have been tested experimentally and found not to be acceptable to the plum aphid.

Numerous species of parasites and predators attack the plum aphid. Syrphid larvae predominate in Placer County, coccinellids and *Leucocoris* in San Joaquin County.

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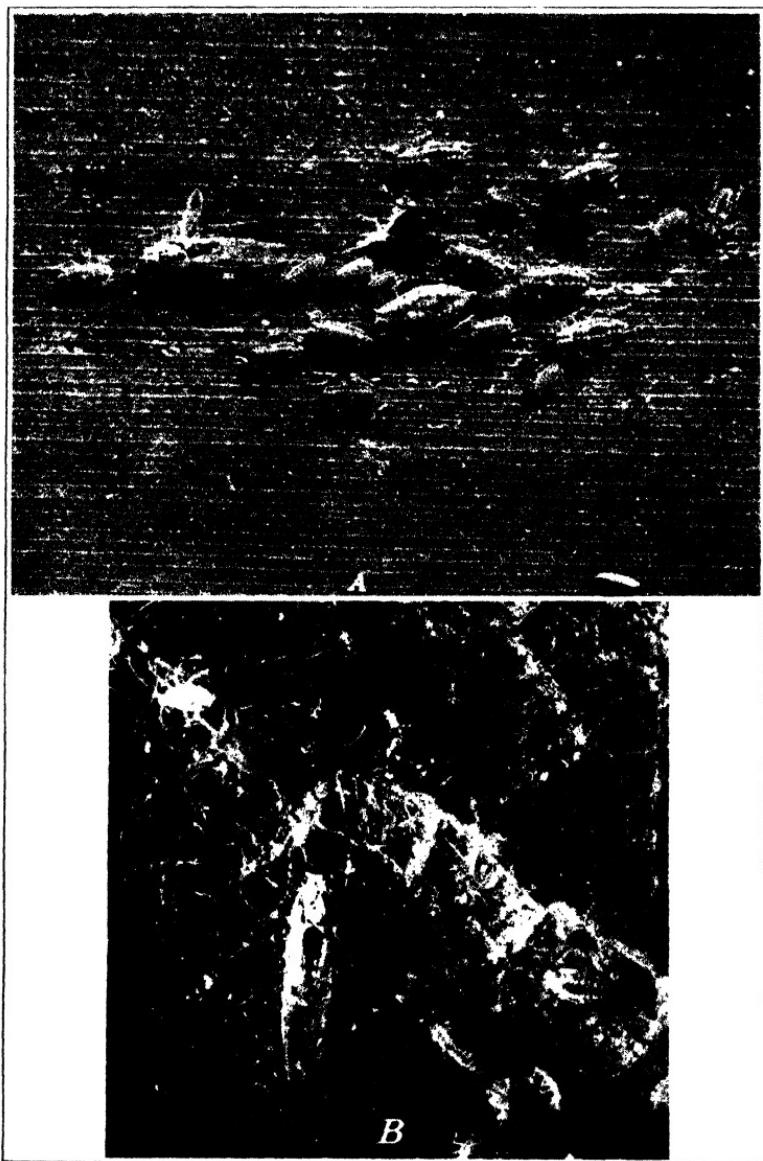


Plate 1.—*A*, Young colony on leaf of cattail. The migrans (alate) has given birth to a number of alienicolae. One of these (largest apterous specimen) is mature and has borne a few young, which are located directly in rear of the alienicola. Note specimen molting, newly molted specimen without mealy covering, and shell of syrphid egg at lower right.

*B*, Gynopara and young oviparous females on plum leaf.



# H I L G A R D I A

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## SYMPTOMATIC AND ETIOLOGIC RELATIONS OF THE CANKER AND THE BLOSSOM BLAST OF PYRUS AND THE BACTERIAL CANKER OF PRUNUS<sup>1</sup>

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### INTRODUCTION

IN 1931 a heretofore undescribed bacterial canker of pear trees was found in Sierra Nevada foothill orchards. A brief discussion of symptoms, giving results of inoculations and comparing the disease with fire blight, *Erwinia amylovora* (Burrill) Bergey *et al.*, was published in 1934.<sup>(18)</sup> The causal organism was not described except as it differed from *Erwinia amylovora* in producing a greenish pigment on many media, thus allying itself with *Phytomonas cerasi* (Griffin) Bergey *et al.*, cause of the stone-fruit bacterial canker.<sup>(19)</sup>

A blossom blast of pear in California differing from that caused by fire blight was briefly described by Thomas and Ark,<sup>(15)</sup> who report the causal organism as similar to those of citrus blast and stone-fruit canker.

The orchards in which the writer first found the limb-canker disease have remained free of blossom blast, though planted with Beurre Bosc, a variety elsewhere susceptible to blossom infection. Limb and blossom symptoms in the trees growing in other districts indicate that all are phases of the same disease. One purpose of this work, therefore, was to compare the bacteria obtained from these parts of the host.

Reports from New York<sup>(a, 4)</sup> and Arkansas<sup>(b, 10)</sup> regarding infection of pear leaves, fruit, and blossoms by bacteria possessing cultural char-

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<sup>a</sup> Superscript numbers in parentheses refer to Literature Cited at end of this paper.

<sup>b</sup> Thomas and Ark designated the blossom disease "blast" to distinguish it from that caused by *Erwinia amylovora*. Rosen<sup>(10)</sup> in Arkansas had used the term earlier to designate a pear-blossom blight caused by an organism similar to *Phytomonas citripulae* (O. O. Smith) Bergey *et al.*, the cause of citrus blast.

acteristics similar to these organisms were additional reasons for the study.

Evidence obtained earlier<sup>(18)</sup> pointed to similarities between the California pear-canker organism and *Phytomonas cerasi*, cause of the stone-fruit canker. This study, therefore, also includes symptomatic and etiologic comparisons of the two diseases.

#### COMPARISON OF THE PEAR AND STONE-FRUIT DISEASES

The bacterial canker of stone-fruit trees is described in detail in an earlier publication.<sup>(17)</sup> Pear blossom blasts and limb cankers are briefly described by Thomas and Ark<sup>(19)</sup> and by the present writer.<sup>(18)</sup> Certain features of the pear disease not previously discussed are included herein.

**Twig Infection.**—In certain years an infection of small branches and twigs causes a considerable loss (fig. 1, *A* and *B*). Sloughing away of the periderm and a spongy condition of the cortex and outer phloem are characteristic symptoms present in all bark cankers whether in large or in small branches. Although differing in external appearance, the twig cankers of the pear and stone-fruit diseases have a similar manner of involving the tissues of the bark. This point will be discussed later.

**Dormant-Bud Blast.**—Figure 1, *C* and *D*, shows small lesions surrounding dormant buds. Both blossom and leaf buds are susceptible to infection and are points from which the disease enters and kills small branches. *Phytomonas cerasi* causes a similar infection of the dormant buds of stone-fruit trees. Twigs as well as small branches are killed by the extension of these infections.

**Infection of the Fruit-Cluster Base.**—A phase of the pear disease that has no counterpart in the stone-fruit disease is the infection of the fruit-cluster bases. Although the exact time of infection is not known, presumably the bacteria enter the fruit-stem scars after the fruit is picked.

**Limb Cankers.**—Branch cankers, adequately pictured in the earlier article,<sup>(18)</sup> were the most common symptoms in the pear orchards where the disease was first found. New cankers and the active margins of old ones present the same appearance as the twig infection shown in figure 1, *A* and *B*. The centers of old cankers are characterized by a longitudinal and transverse cracking of the periderm and by a gradual sloughing away of affected cortex. In many cases where the disease does not at once extend to the cambium, the underlying healthy tissue, in forming a new periderm, forces the diseased tissue outward. Branches diseased for a few years will, therefore, become roughened before natural longitudinal suturing begins. Figure 2 shows a tree in which the bark of certain limbs is rough whereas that of others is smooth.

Branch cankers of the pear and stone-fruit diseases have very similar internal characteristics. In both cases the margins of the affected area are made up of numerous, loosely knit streaks, the paths along which

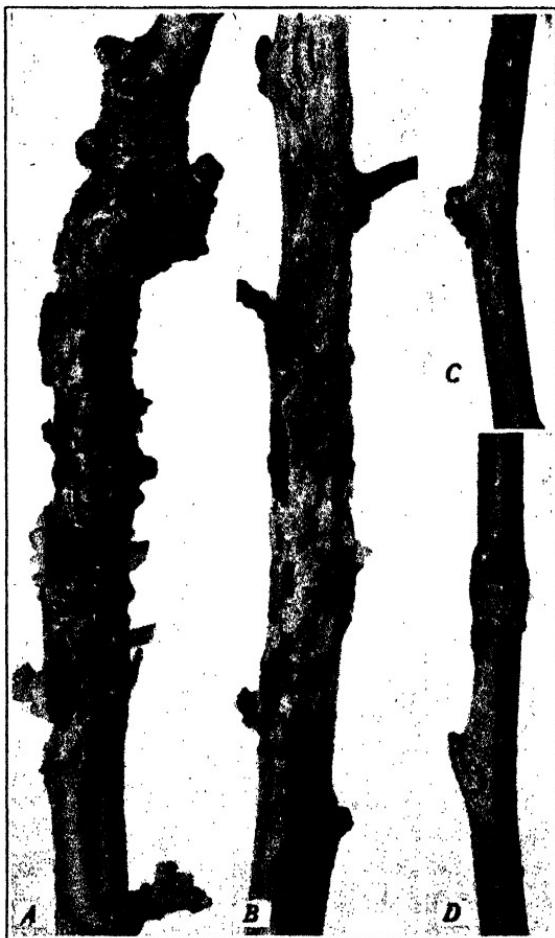


Fig. 1.—Bacterial canker on small branches of pear: *A* and *B*, young terminal shoots with characteristic sloughing away of periderm; *C* and *D*, infection of dormant buds and accompanying lesions in the twig.

bacterial invasion has progressed. On both hosts the streaks of active cankers are light brown and water-soaked. When inactive the streaks are dark brown to black on pear and brown to reddish brown on stone fruits.

*Blossom Blast.*—Thomas and Ark<sup>(15)</sup> found that the pear blossom blast closely resembled blight, caused by *Erwinia amylovora*, but is distinguishable because blast seldom extends more than 1 to 2 inches into the



Fig. 2.—Bacterial canker on trunk and scaffold branches of Wilder pear. The longitudinal and transverse cracking of periderm does not occur on all the branches.

spur and never involves a bacterial exudate. Figure 3 of the present article shows the withered, blackened blossoms and leaves of an infected spur. Arrows indicate the limits of the canker in the branch.

Cherry and apricot blossoms are sometimes blighted by *Phytomonas cerasi*,<sup>(16)</sup> the general symptoms being the same as those of pear blast.

*Leaf and Fruit Infection.*—The disease has not been observed on pear leaves in California, but apricot and cherry leaves are frequently attacked by *Phytomonas cerasi*.

No natural infection of pear fruit has been noted in California. Fruit infection by inoculation has, however, been obtained (fig. 6). In the only case of fruit infection by *Phytomonas cerasi* definitely established, small,

superficial, black pits appeared on the surface of green apricots. Infection has, however, been secured on apricot and plum fruit by needle-puncture inoculations with *P. cerasi*, the resulting symptoms being small, black, sunken pits like those from natural infection.



Fig. 3.—Blossom blast of pear. Arrows point to the margins of the diseased area in the twig. This is usually as far as blossom blast progresses the first year.

*Other Points of Similarity Between the Pear and Stone-Fruit Diseases.*—The pear canker disease differs from fire blight in being active during fall, winter, and early spring, when blight is inactive.<sup>(16)</sup> A similar seasonal nature of stone-fruit bacterial canker has been observed.

To summarize: the pear and stone-fruit diseases possess marked similarities in the parts of trees they attack, in appearance of the invaded tissue, and in their activity during the same seasons of the year.

## PATHOGENICITY STUDIES

The primary object of the inoculation work was to compare bacteria from the pear canker and blossom blast with the organism of stone-fruit canker (*Phytomonas cerasi*). Incidentally, certain similar organisms attacking the pear in other sections of the country, together with the citrus-blast bacterium (*P. citriputeale*), were included in these tests. The history of the cultures, so far as available, is given in table 1.

TABLE 1  
HISTORY OF CULTURES USED IN INOCULATION AND CULTURAL EXPERIMENTS

Organism or culture number	Isolation history		Source of culture		
	Isolated by	Date	State	Host	Diseased part of host
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.....	Author	Aug. 8, 1930	Calif.	Plum	Limb canker
<i>Phytomonas cerasi</i> var. <i>prunicola</i> II.....	Author	Mar. 21, 1932	Calif.	Apricot	Blossom blight
<i>Phytomonas cerasi</i> I.....	Author	Feb. 22, 1930	Calif.	Apricot	Limb canker
<i>Phytomonas cerasi</i> II.....	Author	Apr. 10, 1933	Calif.	Peach	Limb canker
Wilder I.....	Author	Jan. 1, 1931	Calif.	Pear	Limb canker
Wilder II.....	Author	Mar. 7, 1933	Calif.	Pear	Blight of blossom base
Wilder III.....	Author	Aug. 12, 1932	Calif.	Pear	Limb canker
Bartlett I.....	P. A. Ark	May 15, 1932	Calif.	Pear	Blossom blight
Winter Nelis I.....	P. A. Ark	Apr. 24, 1933	Calif.	Pear	Blossom blight
Winter Nelis II.....	P. A. Ark	Apr. 24, 1933	Calif.	Pear	Twig blight
Apple I.....	Author	Apr. 28, 1933	Calif.	Apple	Limb canker
Apple II.....	Author	Apr. 28, 1933	Calif.	Apple	Limb canker
<i>Phytomonas utiformica</i> "r".....	F. Clara	July, 1931	N. Y.	Pear	Blossom blight
<i>Phytomonas utiformica</i> "f".....	F. Clara	July, 1931	N. Y.	Pear	Blossom blight
Arkansas I.....	H. R. Rosen	.....	Ark.	Pear	Blossom blight
Arkansas II.....	H. R. Rosen	.....	Ark.	Pear	Blossom blight
<i>Phytomonas citriputeale</i> .....	Author	Mar. 14, 1932	Calif.	Orange	Twig blast
<i>Phytomonas papulans</i> .....	J. W. Roberts	.....	East.	U. S.	Apple
					Target canker

Eighteen different cultures were employed at various times. Two were cultures of *Phytomonas cerasi* var. *prunicola*: two were *P. cerasi*; and three were cultures the author had isolated from limb cankers of Wilder pear from an orchard in Placer County (Wilder I, II, III). Three (Bartlett I, Winter Nelis I and II) were furnished by P. A. Ark, who had obtained them from blossom and twig blight of pears from El Dorado, San Benito, and Santa Cruz counties, respectively. Two cultures from apple were obtained from Mendocino and Sonoma counties. The two of *Phytomonas utiformica* Clara (isolates "r" and "f") were sent by Clara "to Harvey E. Thomas of this Station. Unfortunately, isolate "r" was lost after the first two series of inoculations in 1933. H. R.

Rosen<sup>(10)</sup> furnished two cultures designated "receptacle" and "petal" (Arkansas I and II, respectively). The culture *P. citriputeale* was obtained from twig blast of orange in Placer County. Inoculations of this organism into lemon and orange fruits produced the sunken lesions typical of the black-pit disease in nature. John W. Roberts furnished the culture that he provisionally called *P. papulans*,<sup>(11)</sup> the organism originally described by Rose<sup>(12)</sup> as the cause of apple blister spot. Roberts obtained this culture from the target canker of apples.

Inoculations were made by first piercing the bark tangentially, then injecting into the holes a drop of the organism in water suspension.

TABLE 2  
RESULTS OF INOCULATING WILDER PEAR TREES JANUARY 20, 1933

Organism	Inoculations producing symptoms, per cent	Length of cankers, mm*
<i>Phytonomas cerasi</i> var. <i>prunicola</i> I.....	77	15- 25†
Wilder I.....	92	25-102
<i>Phytonomas utiformica</i> "r".....	88	25- 64
<i>Phytonomas utiformica</i> "f".....	100	25-127
Controls.....	5‡	5- 10

\* Measurements made 82 days after inoculation.

† Later observations showed that these cankers continued to extend until they involved considerably more area.

‡ Three infections of control wounds on one tree were clearly cases of secondary infections. The remaining 61 wounds were not infected.

From 50 to 75 inoculations were made from each culture used in each experiment into three to five different trees. Control punctures were made in different limbs of the same trees.

Although most of these organisms are similar in certain respects, some difference of opinion exists as to the closeness of the relation. The literature on this phase will be reviewed in a later section.

*Results of Inoculations.*—Table 2 shows the results of inoculations into pear during late winter of 1933. Cultures Wilder I and *Phytomonas utiformica* ("r" and "f") produced extensive cankers (fig. 4) in every way typical of those in nature. *P. cerasi* var. *prunicola* had produced small but definite cankers (fig. 5, C and D). As later observations showed, these cankers continued to extend and eventually became 6 or more inches long. A second series of inoculations made with Wilder I, *P. cerasi*, and *P. cerasi* var. *prunicola* into Wilder pear resulted in cankers from 3 to 6 inches long in all cases. No differences could be found between cankers produced by *P. cerasi* and Wilder I.

In January, 1933, Blenheim apricot limbs and Phillips Cling peach limbs, inoculated with the stone-fruit organisms, Wilder I culture, and



Fig. 4.—Symptoms produced by *Phytoponas utiformica* "r" (A), by *P. utiformica* "f" (B), and by Wilder I (C), on Wilder pear branches. Compare these symptoms with those from natural infections shown in figure 1.

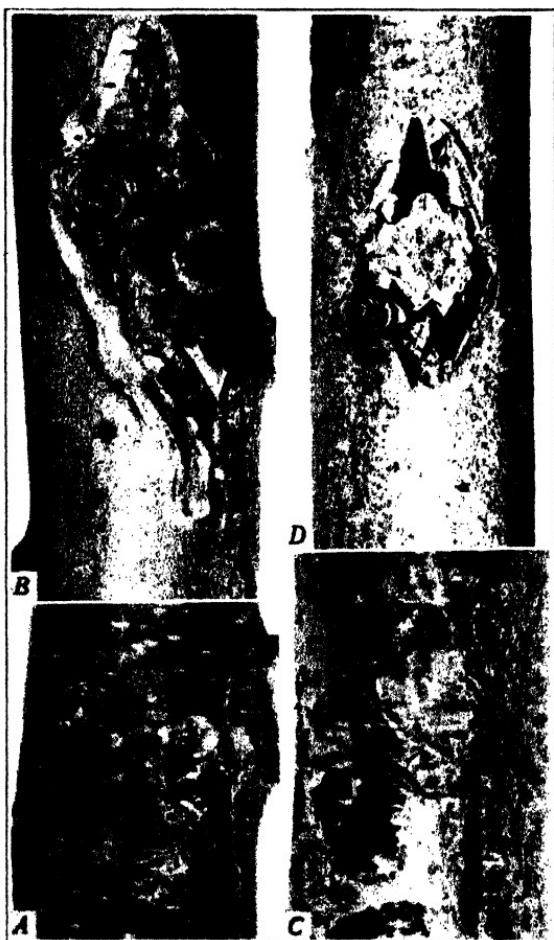


Fig. 5.—Cankers produced by culture Wilder I on Wilder pear (B) and Blenheim apricot (A). On the apricot, the bark canker was  $1\frac{1}{2}$  inches long. C and D are cankers produced by *Phytoponas cerasi* var. *prunicola* on Wilder pear.

the two cultures of *Phytomonas utiformica*, developed definite cankers in every way typical of the bacterial canker of these hosts in nature (table 3). The fact that Wilder I produced somewhat smaller cankers than the stone-fruit organism may or may not be significant.

In February, 1934, when a series of inoculations were made into Duarte plum, conditions apparently did not favor rapid canker development, since cultures of *P. cerasi* and *P. cerasi* var. *prunicola* produced

TABLE 3  
RESULTS OF INOCULATING APRICOT AND PEACH TREES JANUARY 7, 1933

Organism	Blenheim apricot		Phillips Cling peach	
	Inoculations producing symptoms, per cent	Length of cankers,* mm	Inoculations producing symptoms, per cent	Length of cankers, * mm
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.....	82	20-64	60	20-40
<i>Phytomonas cerasi</i> I.....	93	20-30	56	20-50
Wilder I.....	48	20-40	42	20-30
<i>Phytomonas utiformica</i> "r".....	90	20-70	78	10-50
<i>Phytomonas utiformica</i> "l".....	54	20-30	..	..
Controls.....	0	0	0	0

\* Measurements made 26 days after inoculation.

rather small lesions. Nevertheless, as table 4 shows, distinct symptoms were produced by the six cultures obtained from blossom blast, twig blight, and limb canker of pear in California. At least one culture from apple (Apple I), the culture of *Phytomonas utiformica*, and culture Arkansas I also produced distinct symptoms.

On January 21, 1935, inoculations were made into apricot, peach, sweet cherry, and plum. Eleven cultures were used, each being inoculated at 60 places on three trees of each species. As table 5 shows, on apricot and peach all cultures except *Phytomonas papulans* produced diseased areas as large as *P. cerasi* var. *prunicola* or larger. On cherry the diseased areas were smaller, but were otherwise indistinguishable from those produced by the stone-fruit organism. On plum, *P. citriputeale* and culture Winter Nelis I produced cankers somewhat larger than did *P. cerasi* var. *prunicola*, whereas the rest of the cultures, except *P. papulans*, produced cankers somewhat smaller. Thus, *P. papulans* was the only culture that was distinctive on these four hosts. Secondary infections, which occurred on apricot and which will be discussed later, gave additional evidence of the pathogenic abilities of certain pear cultures.

On December 19, 1935, *Phytomonas cerasi* var. *prunicola*, *P. cerasi*,

TABLE 4  
RESULTS OF INOCULATING DUARTE PLUM TREES FEBRUARY 13, 1934

Organism	Inocula-tions producing symp-toms, per cent	Length of cankers, <sup>*</sup> mm	Organism	Inocula-tions producing symp-toms, per cent	Length of cankers, <sup>*</sup> mm
<i>Phytononas cerasi</i> var. <i>pruni-cola</i> I.....	86	5-25	Bartlett I.....	86	5-44
<i>Phytononas cerasi</i> var. <i>pruni-cola</i> II.....	86	10-20	Winter Nellis I.....	79	10-34
<i>Phytononas cerasi</i> I.....	92	5-30	Winter Nellis II.....	83	5-25
<i>Phytononas cerasi</i> II.....	100	20-30	Apple I.....	79	5-25
Wilder I.....	86	5-16	Apple II.....	39	5-10
Wilder II.....	79	5-20	<i>Phytononas utiformica</i> "f".....	66	5-25
Wilder III.....	79	10-25	Arkansas I.....	73	5-22
			Arkansas II.....	79	5-20
			Controls.....	0	0

\* Measurements made 35 days after inoculation.

TABLE 5  
RESULTS OF INOCULATING BLENHEIM APRICOT, PHILLIPS CLING PEACH, LAMBERT CHERRY, AND GRAND DUKE PLUM TREES ON JANUARY 21, 1935

Culture	Apricot		Peach		Cherry		Plum	
	Inocula-tions producing symp-toms, per cent	Average length of cankers, <sup>*</sup> mm	Inocula-tions producing symp-toms, per cent	Average length of cankers, <sup>*</sup> mm	Inocula-tions producing symp-toms, per cent	Average length of cankers, <sup>*</sup> mm	Inocula-tions producing symp-toms, per cent	Average length of cankers, <sup>*</sup> mm
<i>Phytononas cerasi</i> var. <i>pruni-cola</i> .....	70	15	53	15	84	53	75	35
<i>Phytononas uti-formica</i> .....	91	24	83	23	60	32	70	14
<i>Phytononas citri-pudale</i> .....	74	17	60	30	60	26	60	43
<i>Phytononas papu-lans</i> .....	0	0	0	0	0	0	0	0
Wilder I.....	70	14	67	17	48	24	30	15
Wilder II.....	64	20	38	25	47	23	45	13
Winter Nellis I.....	80	18	100	27	80	30	90	40
Winter Nellis II.....	66	19	50	15	50	16	20	13
Apple I.....	56	18	43	19	72	29	30	22
Arkansas I.....	87	25	74	21	72	32	65	25
Arkansas II.....	90	31	53	24	75	35	50	18
Controls.....	0	0	0	0	0	0	0	0

\* Twenty inoculations into each of three trees. The measurements represent the average length of diseased areas along cambium and in bark 40 days after inoculation.

and *P. prunicola* Wor. were each inoculated at 100 places in five Duarte plum trees. Since the temperature did not favor rapid disease development during December and January the cankers produced averaged only 13 millimeters by January 19. Observations on March 4, however, showed that *P. prunicola* had produced cankers as extensive as had the other two organisms, some being 65 millimeters long and 30–35 millimeters wide.

On March 4, 1936, Wilder I and Winter Nelis I were inoculated into young Wilder pear trees. On the same date *Phytomonas cerasi* var. *prunicola*, culture Wilder I and culture Winter Nelis I, were inoculated into Bing sweet cherry. On March 9 examinations showed that in all cases the organisms were invading the bark tissues of both pear and cherry above and below the inoculation points. By March 14 the invaded zones in the pear trees were from 15 to 20 millimeters long and 10 to 15 millimeters wide. No difference existed in symptoms produced by cultures Wilder I and Winter Nelis I. On cherry the three cultures (*P. cerasi* var. *prunicola*, Wilder I, and Winter Nelis I) had produced diseased areas somewhat larger than on pear. Culture Winter Nelis I and *P. cerasi* var. *prunicola* produced somewhat larger cankers than did culture Wilder I, the average lengths being 31, 27, and 21 millimeters respectively.

Table 6 condenses the inoculation data to permit comparisons between results of different years. Whenever a particular culture was listed as pathogenic to a particular host, the character of the symptoms produced was considered in addition to the data on measurements. If on apricot, for example, the symptoms produced by a pear culture were not comparable with those caused by *Phytomonas cerasi* and *P. cerasi* var. *prunicola*, the results were listed as doubtful. This point is stressed because some cultures from pear produced on stone-fruit trees typical bacterial canker symptoms,<sup>13</sup> although the diseased areas were not always so large as those produced by *P. cerasi*.

The California cultures from pear limbs and culture Winter Nelis I from pear blossoms were pathogenic to the five species of stone fruits and to pear. The California cultures from pear blossoms were pathogenic to the stone fruits with but one exception, that of culture Winter Nelis II on European plum and on peach. A culture from apple (Apple I) was pathogenic to Japanese plum and apricot; but inoculations into European plum, peach, and sweet cherry were doubtful. A second culture (Apple II) was doubtful on Japanese plum, the only host into which it was inoculated. The Arkansas cultures were pathogenic to the five stone fruits. *Phytomonas utiformica*, in addition to pear, was pathogenic to

Japanese plum, peach, apricot, and sweet cherry; but doubtful results were obtained on European plum. *Phytomonas citriputeale* produced positive results on European plum, apricot, peach, and sweet cherry. No trials were made on pear. *Phytomonas cerasi* and *P. cerasi* var. *prunicola* were pathogenic to the stone fruits and also produced cankers in pear. *Phytomonas papulans*, on the other hand, produced no symptoms on any of the stone fruits.

TABLE 6  
SUMMARY OF PATHOGENICITY STUDIES\*

Culture	Source of culture		Japanese plum†	European plum	Apricot	Peach	Sweet cherry	Pear
	State	Host						
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I	Calif.	Plum	+	+	+	+	+	+
<i>Phytomonas cerasi</i> var. <i>prunicola</i> II	Calif.	Apricot	+	++	++	++	++	..
<i>Phytomonas cerasi</i> I	Calif.	Apricot	+	++	++	++	++	+
<i>Phytomonas cerasi</i> II	Calif.	Peach	+	++	++	++	++	..
<i>Phytomonas uniformica</i> "f"	N. Y.	Pear	+	±	+	+	+	+
<i>Phytomonas uniformica</i> "r"	N. Y.	Pear	..	..	+	..	..	+
<i>Phytomonas citriputeale</i>	Calif.	Orange	..	+	+	+	+	..
<i>Phytomonas papulans</i>	East. U. S.	Apple	..	-	-	-	-	..
Wilder I	Calif.	Pear	+	+	+	+	+	+
Wilder II	Calif.	Pear	+	+	+	+	+	+
Wilder III	Calif.	Pear	+	..	..	..	..	+
Bartlett I	Calif.	Pear	+	..	..	..	..	..
Winter Nelis I	Calif.	Pear	+	+	+	+	+	+
Winter Nelis II	Calif.	Pear	+	±	+	±	+	..
Apple I	Calif.	Apple	+	+	+	±	±	..
Apple II	Calif.	Apple	±	..	..	..	..	..
Arkansas I	Ark.	Pear	+	+	+	+	+	..
Arkansas II	Ark.	Pear	+	+	+	+	+	..

\* Key to symbols: + = pathogenic; - = nonpathogenic; ± = doubtful.

† This variety of plum (Duarte) is said to be *Prunus (salicina × munsoniana) × salicina*.

‡ These cultures had proved pathogenic to European plum, apricot, peach, and cherry in earlier tests.

**Evidence of Natural Spread of Disease from One Host to Another.**—A host that will develop symptoms when inoculated with bacteria pathogenic to another host will not necessarily contract the disease under field conditions. Evidence, however, points toward the spread of bacteria from stone-fruit trees to pear trees. In one pear orchard, for example, blossom blast occurred only adjacent to three badly diseased apricot trees. The blast was most abundant near the apricots, but was absent a few rows away. In another case, two-year-old pear trees adjacent to old diseased peach trees developed limb cankers, while those farther away remained healthy. One of the worst cases of pear blast observed occurred in a pear orchard interplanted with plums. The plums had apparently suffered from bacterial canker for some years. Although few limb

cankers were present in the pears, for several years blossom blast was prevalent, a fact indicating that the holdover source might have been the plums.

In connection with the inoculation results presented in table 5, attention was called to the development of secondary infections in apricot limbs inoculated with the following pear cultures: Wilder I and II, Winter Nelis I, and Arkansas II. The secondary infection probably did not result from bacteria coming from other trees, since the lesions occurred only below the inoculation points and since control punctures made at one side and above these limbs remained healthy. Healthy trees, furthermore, occurred to windward of those inoculated.

The following observation regarding possible spread of bacteria from pear to citrus should be recorded. In 1932, within two weeks after numerous new cankers appeared in an orchard of Wilder pears, typical citrus blast appeared in a row of orange trees bordering the orchard. No blast had occurred in these orange trees within the preceding three or four years; and close examination after the outbreak in 1932 failed to show any recognizable blast symptoms of previous years.

### CULTURAL TESTS

Unless otherwise stated, the following tests were made at 25° C. All organisms made good growth at this temperature.

The synthetic medium used in most of the carbohydrate tests was the same as that designated basal medium 2 in an earlier article.<sup>17</sup> Its constitution was as follows: potassium dihydrogen phosphate 1.0 gram, magnesium sulfate 0.5 gram, potassium chloride 0.5 gram, sodium nitrate 2.0 grams, and ferrous sulfate 0.01 gram per liter. The pH was adjusted to 6.8 to 7.0 with sodium hydroxide.

*Degree of Fluorescogenesis as a Distinguishing Feature.*—The relations between the pathogenic bacteria that produce a green fluorescence were studied by Burkholder,<sup>18</sup> who attempted to adduce from his own and others' studies the degree of cultural homogeneity exhibited by species included in Bergey's genus *Phytomonas*. In many respects the fluorescent species constituted a closely related group, having common characteristics other than fluorescence. Burkholder's work encouraged Clara<sup>19</sup> to bring together and to study under the same conditions the cultural and pathogenic attributes of many of these species. Clara's conclusions will be reviewed later; he, as well as others, considers fluorescogenesis a cardinal diagnostic character.

The present author's study<sup>17</sup> of the stone-fruit canker indicated that the bacteria involved fell into two types or, as was finally concluded,

two strains or varieties. The type more commonly found on plum, cherry, and apricot differed from the less common type in not producing pigment on potato-dextrose agar. Other slight cultural differences were evident, the consistency of which will be considered later. The less common type was regarded as *Phytomonas cerasi* Griffin, while the more common was designated *P. cerasi* var. *prunicola* n. var.

While information was being secured on the presence of the pear bacterial canker in various localities, the large number of cultures obtained were seen to vary in their ability to produce pigment on potato-dextrose agar. Representative cultures, consequently, were compared with those from stone fruits in the following manner: The cultures were first grown for 24 hours in beef-extract broth and were then transferred to tubes of potato-dextrose agar. After a period extending to 27 days in some cases transfers were again made to beef-extract broth, and after 24 hours to potato-dextrose agar. In all, six such transfers to potato-dextrose agar were made. As the results showed, the organisms from pear separated themselves in the same manner as did the stone-fruit cultures. They consistently did or did not produce a green pigment on this medium. Of the 12 cultures from pear, 4 produced pigment and 8 did not. Of the 13 cultures from stone fruits, 5 produced pigment and 8 did not. The two cultures from apple (Apple I and Apple II) did not produce pigment, nor did those of *Phytomonas utiformica*, *P. citriputeale*, *P. prunicola* (Wormald) Bergey *et al.*, and *P. papulans*. Among those that produced pigment there was some variation; culture Wilder I, for example, produced a clear yellowish-green fluorescence in the medium, similar in every respect to that of *P. cerasi* I, whereas culture Wilder II produced at first a clear yellowish-green fluorescence, but after a few days a brownish discoloration of the agar. The same type of variation existed between *P. cerasi* I and *P. cerasi* II.

In order that later reference may be made to the ability of the individual cultures used in the inoculation experiments to produce pigment in potato-dextrose agar, the following list is given:

<i>Fluorescent</i>	<i>Nonfluorescent</i>	
Wilder I	Bartlett I	<i>Phytomonas citriputeale</i>
Wilder II	Winter Nelis I	<i>Phytomonas utiformica</i>
Wilder III	Winter Nelis II	<i>Phytomonas prunicola</i> Wor.
	Apple I	<i>Phytomonas papulans</i>
	Apple II	Arkansas I
		Arkansas II

The stone-fruit cultures designated *Phytomonas cerasi* are, of course, fluorescent, and those designated *P. cerasi* var. *prunicola* are nonfluorescent on potato-dextrose agar.

The difference in pigment production that distinguished *Phytomonas cerasi* and *P. cerasi* var. *prunicola* on potato-dextrose agar was not so clear-cut on certain other media,<sup>10</sup> being more a difference of intensity than of quality. Thus, in a synthetic liquid medium containing mannitol, glycerol, or sodium succinate as the energy source, a greenish-yellow pigment was produced by both, although that produced by *P. cerasi* was more intense. When cultures Wilder I, *P. cerasi*, *P. cerasi* var. *prunicola*, and *P. utiformica* were grown comparatively in the presence of various carbon sources (23 in all), culture Wilder I resembled *P. cerasi*, while *P. utiformica* resembled *P. cerasi* var. *prunicola* in fluorescence.

*Carbohydrate Utilization.*—The medium used in these tests was basal medium 2, described earlier in this section. It soon proved ill-adapted to studies of increase in hydrogen-ion concentration when the carbon source used was not readily utilized by the bacteria. Trehalose and raffinose, for example, are less readily utilized than dextrose; yet growth is moderately luxuriant after about one week. If basal medium 2 is used, the pH is unchanged or is slightly increased. If, on the other hand, the basal medium is that proposed by the Society of American Bacteriologists *Manual*,<sup>11</sup> in which the nitrogen source is monobasic ammonium phosphate, the bacteria produce a definite decrease in pH when utilizing trehalose and raffinose. As will be mentioned later, a substitution of ammonium sulfate for sodium nitrate in basal medium 2 permits the hydrogen-ion concentration to increase more rapidly in the presence of dextrose as an energy source. Since the pH change is determined not only by the hydrogen or hydroxyl ions derived from the carbon source, but by the ions derived from all other constituents of the medium and by the buffering effects of the constituents, the conditions of the test must be specified. The basal medium used for the carbon-utilization tests reported in table 7 was basal medium 2. Despite the objectionable features of this medium in the presence of a poorly utilized carbon source, it supported a luxuriant growth and favored development of the fluorescent pigment. For those reasons it was used extensively.

As shown in table 7, the pear-canker organism (Wilder I) was grown comparatively with *Phytomonas cerasi* var. *prunicola*, *P. cerasi*, *P. prunicola* Wor., and *P. utiformica* on basal medium 2 in the presence of twenty-three carbon sources. *Phytomonas utiformica* was grown on all but three of these carbon compounds. The plus and minus signs represent greater acidity and greater alkalinity than the control tube after the bacteria had been growing for 10 days at 25° C. With but one exception the organisms produced the same type of reactions on all carbon

sources. The exception was in the case of rhamnose, where culture Wilder I had made visible growth although the others had not. This difference is not significant, since in further tests the other cultures produced visible growth after 14 days or so.

In later tests all the cultures used in the pathogenicity studies were grown comparatively on basal medium 2 with xylose, dextrose, and

TABLE 7  
CARBOHYDRATE UTILIZATION\*

Culture	Carbon source																							
	Xylose	Arabinose	Mannose	Dextrose	Levulose	Galactose	Sucrose	Maltose	Lactose	Trehalose	Raffinose	Rhamnose	Mannitol	Glycerol	Peptone	Sodium asparagine	Sodium succinate	Sodium citrate	Sodium malate	Sodium lactate	Sodium tartrate	Sodium acetate	Sodium oxalate	Sodium formate
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	0	0	0	0	0	0
<i>Phytomonas cerasi</i> var. <i>prunicola</i> II.	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
<i>Phytomonas cerasi</i> I.	-	-	-	-	-	-	-	-	-	-	0	+	+	-	-	-	-	-	0	0	0	0	0	0
<i>Phytomonas cerasi</i> II.	-	-	-	-	-	-	-	-	-	-	0	+	+	-	-	-	-	-	0	0	0	0	0	0
<i>Phytomonas prunicola</i> Wor.	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	0	0	0	0	0	0
Wilder I.	+	+	+	+	+	+	-	t	-	-	+	+	+	-	-	-	-	-	0	0	0	0	0	0
Wilder II.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
Wilder III.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
Bartlett I.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
Winter Nelis I.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
Winter Nelis II.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
Apple I.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
Apple II.	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
<i>Phytomonas uniformica</i> I.	+	+	+	+	+	+	-	-	-	-	0	+	+	-	-	-	-	-	0	0	0	0	0	0
Arkansas I.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
Arkansas II.	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
<i>Phytomonas citriputeale</i> .	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
<i>Phytomonas papulans</i> .	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0
<i>Ervinia amylovora</i> .	0	+	+	+	+	+	+	0	+	+	0	0	0	-	0	0	0	0	0	0	0	0	0	0

\* Key to symbols: + = change towards acid side; - = change towards alkaline side; t = growth but no change in pH; 0 = no growth.

sucrose as energy sources (10 grams per liter). With but one exception, that of culture Apple II in the presence of sucrose, all the organisms produced acid from the sugars (table 7). Although culture Apple II made a good growth on sucrose, no change in pH was visible after 10 days. In the degree to which the pH was changed, an apparent consistent difference was noticed on sucrose. Thus *Phytomonas cerasi* and the pear cultures that produced pigment on potato-dextrose agar caused less increase in hydrogen-ion concentration than did *P. cerasi* var. *prunicola* and those cultures (except Apple II) that did not produce pigment on potato-dextrose agar. In three experiments, for example, after 5 days the

latter group of cultures had reduced the pH to 3.8-4.1, whereas the former had reduced it only to 6.4-6.6. Under the conditions of these experiments at least, the cultures separated along the same lines as on potato-dextrose agar.

All the organisms were inoculated into tubes of basal medium 2 with formic acid (0.15 per cent, pH 6.8) as the only carbon source. None of

TABLE 8  
MISCELLANEOUS CULTURAL FEATURES\*

Culture	Gelatin liquefaction	Hydrogen sulfide production	Milk		Growth on nitrogen compounds			
			pH change	Peptonization	Ammonium sulfate	Sodium asparaginate	Asparagine	Glycine
<i>Phytomonas cerasi</i> var. <i>prunicola</i> I.....	+	-	Ak	+	+	+	+	+
<i>Phytomonas cerasi</i> var. <i>prunicola</i> II.....	+	-	Ak	-	+	+	+	+
<i>Phytomonas cerasi</i> I.....	+	-	Ak	+	+	+	+	+
<i>Phytomonas cerasi</i> II.....	+	-	Ak	+	+	+	+	+
<i>Phytomonas prunicola</i> Wor.....	+	-	Ak	+	+	+	+	+
Wilder I.....	+	-	Ak	+	+	+	+	+
Wilder II.....	+	-	Ak	+	+	+	+	+
Wilder III.....	+	-	Ak	+	+	+	+	+
Bartlett I.....	+	-	Ak	-	+	+	+	+
Winter Nelis I.....	+	-	Ak	+	+	+	+	+
Winter Nelis II.....	+	-	Ak	+	+	+	+	+
Apple I.....	+	-	Ak	+	+	+	+	+
Apple II.....	-	-	Ak	-	+	+	+	-
<i>Phytomonas utiformica</i> I.....	+	-	Ak	+	+	+	+	+
Arkansas I.....	+	-	Ak	+	+	+	+	+
Arkansas II.....	+	-	Ak	+	+	+	+	+
<i>Phytomonas citripuleale</i> .....	+	-	Ak	+	+	+	+	+
<i>Phytomonas papulans</i> .....	-	-	Ak	+	+	+	+	+
<i>Erwinia amyloëra</i> .....	+	-	...	...	+	+	+	...

\* Key to symbols: + = positive reaction or growth, as pertains to the respective headings; - = negative reaction or no growth; Ak = a shift of pH from 6.2 towards alkaline side.

the organisms made visible growth in this medium even after three weeks at 25° C. Apparently, therefore, formic acid is not an energy source that these bacteria can readily utilize (table 7).

*Nitrogen Source*.—The influence of the nitrogen source on the characteristics of the pH change produced by the bacteria has been mentioned. Sodium nitrate, though supporting an abundant growth, was less conducive to increase in hydrogen-ion concentration than monobasic ammonium phosphate. A further study of nitrogen sources revealed that ammonium sulfate, asparagine, sodium asparaginate, and glycine were utilized by all the bacteria (table 8). The characteristics of the pH change were as follows: on ammonium sulfate a rapid hydrogen-

ion increase occurred within a few days; on sodium nitrate and glycine the increase was much slower; on asparagine and sodium asparaginate the initial pH change was towards the alkaline side and was followed by a reversal only after several days. In the degree of pH change, *Phytomonas papulans* and culture Apple II differed from the rest on sodium nitrate and glycine.

*Nutrient-Dextrose Broth*.—This medium was beef-extract broth to which had been added 10 grams of dextrose per liter. The pH was adjusted to 6.8. By the end of 72 hours all the bacteria except *Phytomonas papulans* and culture Apple II had produced a dense uniform turbidity of the medium and a slight, easily fragmented surface film. *Phytomonas papulans* and culture Apple II differed from the rest in producing a tough, membranous surface film and very little turbidity.

*Colony Characteristics on Potato-Dextrose Agar*.—The consistency, topography, and internal structure of potato-dextrose agar colonies varied greatly even in *Phytomonas cerasi* I, the descendant of a single-cell isolation.<sup>27</sup> On the whole, the colonies of all the cultures except *P. papulans* and culture Apple II were similar, being after 48 hours from 1.5 to 3 millimeters in diameter. The margins were either entire or slightly lobed. The topography was flat or slightly raised. The consistency was butyrous, except as reported earlier for *P. cerasi*;<sup>28</sup> the color slightly bluish to white. The internal structure was amorphous or broken by dark, wavy lines extending in a general radial direction. Wormald<sup>29</sup> has reported this last-named feature to be characteristic of his *P. prunicola*. The colonies of *Phytomonas papulans* and culture Apple II differed from the rest in being more opaque and somewhat slimy.

*Liquefaction of Gelatin*.—Stab cultures were incubated at 21° C., observations being made at 2-day intervals. The cultures of *Phytomonas cerasi* were the first to begin liquefaction, followed by those of *P. cerasi* var. *prunicola* (table 8). By the end of 168 hours all cultures, except Apple II and *P. papulans*, had produced a stratiform liquefaction to a depth of 1 inch or more. The tubes were then placed at 25° C., but culture Apple II and *P. papulans* failed to start liquefaction after 3 days at this temperature.

*Reaction in Milk*.—Enough litmus was added to one lot of skimmed milk to produce a distinct blue. To another lot was added enough brom thymol blue to give a distinct color. The tubes were sterilized by steaming at atmospheric pressure for 20 minutes on 4 successive days. The final pH was approximately 6.2.

In five experiments the initial reaction of all cultures was an increase

in alkalinity (table 8). Clearing of the milk accompanied by a distinct odor of peptonization began in most cases within 4 or 5 days. In this reaction *P. cerasi* was slightly more rapid than the rest. *Phytomonas papulans*, culture Apple II, and culture Bartlett I were distinguishable from the rest by their failure to produce a peptonization after 11 days.

*Hydrogen Sulfide Production*.—Beef-extract agar was prepared as recommended by the Society of American Bacteriologists *Manual*.<sup>10</sup> None of the bacteria under study produced hydrogen sulfide in this medium (table 8).

*Starch Hydrolysis*.—Two types of media were employed for these tests: (1) beef-extract agar plus 10 grams of starch per liter and (2) basal medium 2 plus 10 grams of starch per liter (table 8). Beside *Phytomonas cerasi*, *P. cerasi* var. *prunicola*, and *P. prunicola* the only other culture used was Wilder I. Good growth but no starch hydrolysis was made on the former medium; no growth was made on the latter.

*Malachite Green Agar*.—The growth of the bacteria on malachite green agar is reported because further differentiation of *Phytomonas papulans* and Apple II was obtained. To basal medium 2 were added 10 grams of dextrose, 15 grams of agar, and malachite green (1:100,000); the pH was adjusted to 6.8. In petri dishes this medium was distinctly green.

*Phytomonas papulans* and culture Apple II were differentiated from the rest because *P. papulans* failed to grow and culture Apple II grew only slightly. Although culture Bartlett I and *P. cerasi* var. *prunicola* II grew somewhat more slowly than the rest, they conformed to the characteristics of a majority of the others—namely: (1) a flat, butyrous, opalescent growth, (2) later a greenish-yellow pigment that stained the bacterial mass and diffused into the medium (*P. cerasi* I and II produced pigment earlier and in greater intensity), and (3) gradual disappearance of the malachite green after about 2 or 3 days so that by the end of 10 days the plates were usually cleared of the stain.

*Differentiation of Erwinia Amylovora and the Green-Fluorescent Organisms*.—Since some rapid method of distinguishing *Erwinia amylovora* from the pear-canker organism was desirable, the bacillus was included in the studies of fluorescogenesis and carbon-source utilization. Although the blast and canker cultures produced pigment on basal medium 2 in the presence of a number of carbon compounds, *E. amylovora* showed no indication of producing pigment. According to table 7, under the conditions of these tests, *E. amylovora* was distinguishable from the green-fluorescent organisms when growing on a number of carbon sources. The most rapid method of differentiating these organ-

isms, however, would consist in adding to basal medium 2, glycerol, mannitol, or peptone, carbon compounds especially favorable to pigment production.

Besides cultural methods, figure 6 shows that *Erwinia amylovora* may be distinguished from the fluorescent organisms by inoculations

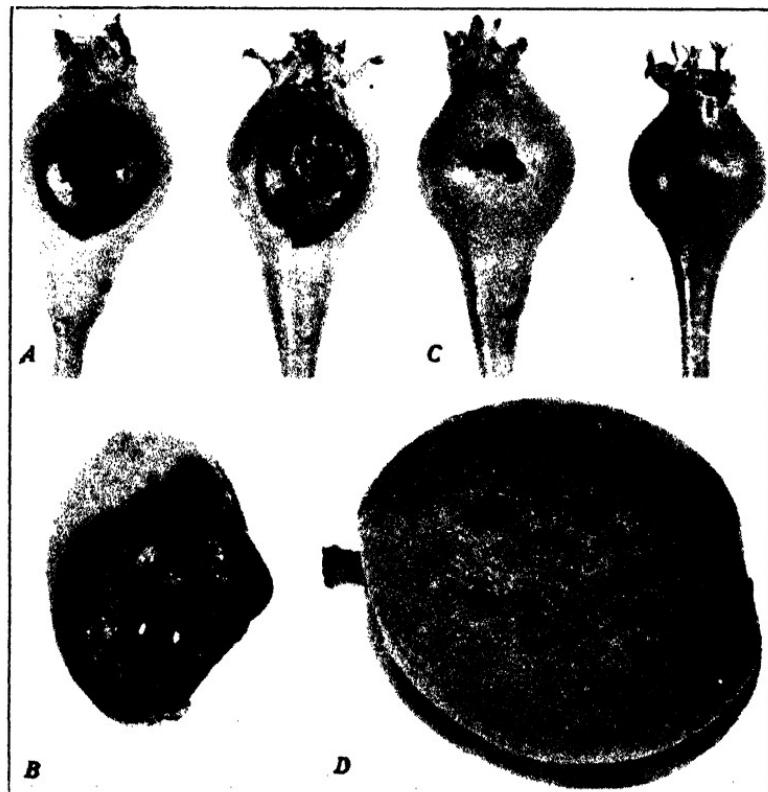


Fig. 6.—Difference in symptoms produced on pear and apricot by *Erwinia amylovora* (A, B) and by the pear-canker and blast organism (C, D). *Phytomonas cerasi* var. *prunicola* produced the same symptoms as did the pear-canker bacterium.

into young pear and apricot fruits. The fire-blight organism readily involved the entire fruit, producing a white, slimy exudate, while the pear-blast and stone-fruit organisms produced around the needle punctures only small, black, sunken pits and no visible exudate. Smith and Fawcett<sup>(13)</sup> have obtained with *Phytomonas citriputeale*, *P. syringae*, and *P. cerasi* the same type of black pits on a variety of fruits.

## DISCUSSION

The literature contains considerable evidence that certain organisms included in this study are closely related. Although much of this evidence has been summarized elsewhere,<sup>(10)</sup> some recapitulation is necessary.

As Bryan,<sup>(11)</sup> Smith,<sup>(12)</sup> and Smith and Fawcett<sup>(13)</sup> have shown, *Phytomonas citriputeale* C. O. Smith is similar to if not identical with *P. syringae* (Van Hall) Bergey *et al.* As the last-named authors further showed, *P. cerasi*<sup>6</sup> is also closely related to these organisms. Elliott<sup>(14)</sup> has since considered *P. citriputeale* but not *P. cerasi* synonymous with *P. syringae*.

Both Smith<sup>(12)</sup> and the present writer<sup>(15)</sup> have questioned the status of *Phytomonas prunicola*, after Wormald's description of it as a distinct species. Smith considered it to be similar to if not identical with *P. citriputeale*, whereas the writer found it identical with an organism he considered to be a strain or variety of *P. cerasi*—namely, *P. cerasi* var. *prunicola*.

In 1932 Clara<sup>(16)</sup> described *Phytomonas utiformica* as the cause of blossom blight, fruit spot, and leaf spot of pears in New York. The same year Rosen<sup>(17)</sup> found a similar blossom disease on pears in Arkansas; and a year later Rosen and Bleeker<sup>(18)</sup> published comparative serological and pathological tests in which they included their organism from pear, a culture of *P. syringae*, and a culture of *P. prunicola*. They concluded that these organisms were identical and questioned the advisability of considering *P. cerasi*, *P. papulans*, *P. nectarophila* (Doidge) Bergey *et al.*, *P. barkeri* (Berridge) Bergey *et al.*, and *P. utiformica* as species separated from the lilac organism, *P. syringae*, by significant differences.

Clara<sup>(16)</sup> compared a number of green-fluorescent organisms, including *Phytomonas cerasi*, *P. utiformica*, and *P. syringae*. On the basis of pathogenicity to seventeen hosts he concluded that these organisms were related, but believed that certain cultural differences warranted separating them into species.

Dunegan<sup>(19)</sup> has recently reported results of inoculating peach with *Phytomonas syringae* Van Hall, *P. prunicola* Wor., *P. mors-prunorum* (Wormald) Bergey *et al.*<sup>(13)</sup> *P. papulans*, a bacterium from apple target canker, and a bacterium from leaf spot of Italian prune. He found that when any of these bacteria were injected into leaves and green shoots, chlorotic or bleached areas surrounded by purplish zones were devel-

<sup>6</sup> Smith and Fawcett's culture of *P. cerasi* was obtained in California. After corresponding with Smith the writer is convinced that it was *P. cerasi* var. *prunicola*. For description of this variety see the writer's earlier publication.<sup>(15)</sup>

oped, symptoms differing from those produced by *P. pruni* (E. F. Smith) Bergey *et al.*, on this host.

We see, therefore, in the works of most of these authors a tendency towards clearing the literature of certain species created as the result of studying each on a limited number of hosts. Such a tendency is justifiable as far as it is founded upon a direct comparison of the organism in question. The interlinking evidence supplied by the works of Bryan,<sup>(1)</sup> Smith and Fawcett,<sup>(12)</sup> Smith,<sup>(13)</sup> Rosen and Bleeker,<sup>(10)</sup> and the writer<sup>(14, 17, 18)</sup> furnishes reasons for considering *P. syringae*, *P. citriputeale*, *P. cerasi* var. *prunicola*, and *P. prunicola* as either identical or differing only to a slight degree. Clara<sup>(6)</sup> alone maintains that *P. syringae* and *P. cerasi*<sup>a</sup> are distinct species, although he considers *P. citriputeale* and a number of other green-fluorescent pathogens synonymous with *P. syringae*.

If we examine Clara's<sup>(6)</sup> reasons for regarding the three species as distinct, we see that they pertain both to pathogenic and to cultural features. He found, for example, that they differed in their ability to produce lesions on such diverse plants as *Trifolium repens*, *Holcus* sp., *Zea mays*, and *Syringa vulgaris*. On thirteen other hosts, including *Pyrus communis* and *Prunus avium*, the three organisms were indistinguishable. His results with *Syringa vulgaris* differ from those of Smith and Fawcett<sup>(13)</sup> inasmuch as he found *P. cerasi* nonpathogenic to this host, whereas Smith and Fawcett obtained infection.

In cultural tests Clara found the three organisms very similar in many respects, but different in their reaction on certain carbon compounds—namely, raffinose, glycerol, salicin, acetic acid, and formic acid. Thus he reported that *Phytomonas utiformica* was the only one to "ferment" raffinose, salicin, and formic acid; that *P. cerasi* and *P. utiformica* but not *P. syringae* fermented acetic acid, whereas *P. utiformica* and *P. syringae* but not *P. cerasi* fermented glycerol. His conclusions regarding the failure of *P. cerasi* to ferment glycerol are contrary to those of Smith and Fawcett<sup>(13)</sup> and of the writer,<sup>(17)</sup> who found this compound to be an excellent energy source. Likewise his findings regarding failure of *P. cerasi* to ferment raffinose and salicin do not conform with the writer's earlier results<sup>(17)</sup> nor with the studies presented herein, which indicate that these compounds supported fair growth. In the present study *P. utiformica* failed to make visible growth on a medium containing formic acid in the same concentration as used by Clara; acetic acid was not used.

As this brief review shows, the majority of the workers consider the organisms used in this study very closely related, and Clara's dissenting

<sup>a</sup> The culture supplied Clara was that of *Phytomonas cerasi* var. *prunicola*.

views are based upon much evidence directly contrary to that of the others.

We may now consider the results of the present work. Though certain gaps exist in the inoculation data and though cultural tests are by no means complete, the studies have yielded certain evidence:

1. The California cultures from pear differed consistently among themselves in one respect—production of fluorescent pigment on potato-dextrose agar. That is, the three cultures from limb canker of Wilder pear produced fluorescence on potato-dextrose agar, whereas those from twigs and blossoms did not, a characteristic similar to that separating *Phytomonas cerasi* from *P. cerasi* var. *prunicola*. The few instances in which the pear organisms varied in other respects could easily have resulted from experimental error.

2. The mutual pathogenic abilities, the parallel fluorescigenic variability on potato-dextrose agar, and the similar reactions in all other tests exhibited by the stone-fruit and California pear cultures give no indication that they are very different.

3. Apple II culture, although belonging to the green-fluorescent group, is definitely different from the pear cultures and from Apple I; the latter appears to be very similar to the pear cultures.

4. The culture furnished the writer by Roberts and provisionally designated by him as *Phytomonas papulans* is distinctly unlike any of the other organisms tested herein. Dunegan,<sup>(5)</sup> so far as known, is the only worker who has compared *P. papulans* with one of those included in the present study.

5. *Phytomonas citriputeale*, *P. utiformica*, and the pear-blast cultures from Arkansas were pathogenically similar to the California pear cultures, and to *P. cerasi* and *P. cerasi* var. *prunicola* when inoculated into five species of stone fruits. *Phytomonas utiformica* was, furthermore, shown to produce the same type of symptoms on pear as did the California pear-canker organism. As far as the cultural studies went, this first-named group of organisms agreed with *P. cerasi* var. *prunicola* and with those California pear cultures that were not fluorescent on potato-dextrose agar.

This summary indicates that the only clear cases of differences within this group of cultures were those of *Phytomonas papulans* and Apple II. This is true as far as both the pathogenic and cultural tests are concerned. The rest of the cultures, however, though identical in inoculation tests, exhibited on media certain differences that should be mentioned. We saw that the three California cultures from pear-limb canker (Wilder I, II, and III), when grown on potato-dextrose agar, in their

production of pigment resembled *P. cerasi*, whereas the remaining California pear cultures as well as *P. citriputeale*, *P. utiformica*, and cultures Apple I and II, in their failure to produce pigment on potato-dextrose agar, resembled *P. cerasi* var. *prunicola*. As earlier comparisons<sup>(17)</sup> had shown, *P. cerasi* and *P. cerasi* var. *prunicola* differed slightly in other tests. When these tests were undertaken for the present study, the similar slight differences were again evident: *P. cerasi*, for example, began to peptonize milk and to liquefy gelatin somewhat earlier than did *P. cerasi* var. *prunicola*. The pear cultures that resembled *P. cerasi* on potato-dextrose agar, on the other hand, were not so distinguished from those that resembled *P. cerasi* var. *prunicola*. Another example of separation of the cultures was afforded by sucrose in basal medium 2. Here *P. cerasi* and the three pear cultures that resembled it on potato-dextrose agar (Wilder I, II, and III) produced a smaller decrease in pH than did *P. cerasi* var. *prunicola* or any of the other cultures except *P. papulans* and culture Apple II.

Hence, except in the cases of *Phytomonas papulans* and culture Apple II, the only consistent separations of cultures were in their fluorescent capacities on potato-dextrose agar and in the degree to which they changed pH in the presence of sucrose. Host source, on the other hand, did not appear to be important as a line of cleavage. Particularly can this be said of the two stone-fruit organisms, of *P. utiformica*, of culture Winter Nelis I, and of culture Wilder I, all of which were inoculated into pear as well as five species of stone fruits. How many more slight differences can be obtained by increasing the number of tests and by refining the technique can only be surmised. Unquestionably, a number of small differences would be regarded by some as justifying the continuation of the names of existing species and the use of new names for those unnamed organisms included herein. The final disposition will, of course, depend upon more complete studies and upon the prevailing conception of species limits.

Provisionally, at least, the evidence justifies including in one species *Phytomonas cerasi*, *P. cerasi* var. *prunicola*, *P. citriputeale*, *P. utiformica*, Rosen's organism, and the California pear organism. These organisms are unquestionably very closely related to *P. syringae*. The fact that there is no recorded variation of *P. syringae* comparable with that separating *P. cerasi* and *P. cerasi* var. *prunicola* cannot be overlooked. If any changes are made, *P. cerasi* should probably retain a varietal rank.

## SUMMARY AND CONCLUSIONS

The major object of this work was to establish the relation between a canker and blossom blast of pear and the bacterial canker of stone-fruit trees. Both the pear canker and pear blossom blast were known to be caused by bacteria that were on standard culture media similar to each other and to *Phytomonas cerasi*, cause of the stone-fruit bacterial canker. By observing the diseases of the two hosts for a number of seasons, information was obtained concerning the season of activity, the parts of the hosts attacked, and the character of the symptoms. To determine pathogenic similarities, the bacteria were inoculated at various times into *Pyrus* sp. and into five species of *Prunus*. By cultural tests the bacteria were compared as to growth on various standard media, growth in special media containing different carbohydrate and nitrogen sources, reactions in milk, liquefaction of gelatin, and production of hydrogen sulfide. Besides the two strains of stone-fruit organisms (*P. cerasi* and *P. cerasi* var. *prunicola*) and bacteria from pear limb cankers and blossom blast, the pathogenicity and cultural studies included the following organisms: *P. utiformica*, *P. papulans*, *P. citriputeale*, cultures obtained by Rosen from pear blossoms in Arkansas, and cultures obtained by the writer from apple in California. Incidentally, *Erwinia amylovora* was carried through certain of the cultural tests, and a method for differentiating it from the canker organisms is described.

The results of these studies afforded the following conclusions: First, the limb canker and blossom blast of pear are phases of the same disease, which also attacks dormant buds, twigs, and fruit. Second, the pear and the stone-fruit diseases exhibit similarities as to parts of the host attacked, character of symptoms, and season of activity. The bacteria from the two hosts were, furthermore, identical in the inoculation and cultural tests. Third, the inoculation and cultural tests support the view that *Phytomonas utiformica*, *P. citriputeale*, and the bacterium furnished by Rosen are identical with the stone-fruit organism. The bacterium recently isolated by Roberts and designated *P. papulans* is an unrelated species.

In the writer's opinion, therefore, these organisms, except of course *Phytomonas papulans*, should be given the same species name. The preponderance of evidence in the literature points towards *P. syringae* as the correct binomial.

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# **INHERITANCE OF RESISTANCE TO POWDERY MILDEW IN BEANS**

**BJARNE DUNDAS**



# INHERITANCE OF RESISTANCE TO POWDERY MILDEW IN BEANS<sup>1</sup>

BJARNE DUNDAS<sup>2</sup>

POWDERY MILDEW (*Erysiphe polygoni* D.C.) frequently causes considerable loss in beans (*Phaseolus vulgaris* L.) grown in the coastal districts of California. Among the numerous bean varieties grown in test plots at Berkeley each season by W. W. Mackie, of the Division of Agronomy, differences in varietal susceptibility have been apparent.

In the summer of 1932 the mildew was very abundant at Berkeley, but the Pinto and some other varieties were not infected. The following winter crosses were made in the greenhouse between these and certain other varieties with the idea of studying the inheritance of resistance and introducing resistance into commercial varieties which lack it.

## METHODS EMPLOYED IN INOCULATION TESTS

The reaction of the bean plants to mildew was determined by inoculating detached leaflets floated on a sugar solution in petri dishes, a method used by Yarwood<sup>3</sup> in his work with the powdery mildew of red clover. This method has numerous advantages. Plants may be grown in the greenhouse and tested at any time of the year, and uniformity of inoculum and environmental conditions during inoculation tests are insured. The same single-spore culture of the mildew was propagated in petri dishes free from contamination by other strains of mildew and was used in all of the inoculation tests, which extended over a period of more than three years.

*Length of Life of the Detached Leaflets.*—Tests made to determine what substrate was most favorable to prolonged life of the detached bean leaflets showed that a 10 per cent solution of sucrose was superior to several higher and lower concentrations tested and to pure water or Hoagland culture solution. In petri dishes leaves floated on a 10 per cent sucrose solution or lying on cotton saturated with this solution remained alive (turgid and normal green in color) two to three weeks at room temperature and over a month at 8° C with a maximum of 55 days at 8°, 12 days at 25°, and 7 days at 31°. Time of day of collection of the

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<sup>4</sup> Superscript numbers in parentheses refer to Literature Cited at end of this paper.

leaflets seemed to be unimportant. Leaves lived about two days longer in light than in the dark. The third youngest unfolded leaf was found to be similar to older leaves in its reaction to mildew and was generally used. In their mildew reaction, leaves from greenhouse plants were similar to those from field plants. Infection with the mildew was found to shorten the life of the detached leaflets by 2 to 8 days.

*Optimum Conditions for Production of Inoculum and for Infection.*—Spores of powdery mildew produced in the light germinated somewhat better than those produced in the dark, and young spores from colonies 3 to 6 days old germinated much better than those from colonies over 19 days old. Spores produced under relative humidities<sup>(a, b)</sup> of 8 per cent (over KOH), 33 per cent (over  $MgCl_2 \cdot 6H_2O$ ) and 79 per cent (over  $NH_4Cl$ ) germinated about equally well and somewhat better than those produced at 0 (over  $P_2O_5$ ) or 100 per cent relative humidity.

It was found that spores would germinate on dry slides as well as on water. Germination of dry spores (25–77 per cent) on dry slides occurred promptly at temperatures between 8° and 25° C, at ordinary room humidities as well as in saturated air and in darkness or in light. In fact, a low percentage of spores germinated at temperatures as high as 28° and 31° C and at room temperature in a relative humidity of 8 per cent. No spores germinated at approximately zero relative humidity.

The time of day when spores were applied to the leaflets made no difference in the resultant growth of mildew. On leaflets on 10 per cent sugar solution the mildew developed equally well in the dark and in the light, but on weaker or stronger sugar solutions it developed better in the light. It developed well at 15° C and at room temperature (about 21°) but less abundantly at 25° and not at all at 31°. At 8°, 22 days were required for it to reach a stage of development such as that arrived at in 8 or 9 days at room temperature. It was found to develop well over a wide range of relative humidity.

*Inoculation of Leaves.*—Inoculum was produced on leaflets on 10 per cent sugar solution in petri dishes stored in the light at room temperatures, and the spores were used when the colonies were 6 to 10 days old.

In order to determine the relative susceptibility of individual plants, the third youngest unfolded leaf was placed in a petri dish on cotton soaked with a 10 per cent sucrose solution with the upper epidermis uppermost. To obtain spores for inoculation, heavily mildewed portions of a leaflet were cut out and held with a forceps over the leaflet to be inoculated, and by tapping the forceps the spores were dislodged. Or the spores were deposited by gently touching the leaflet with the mil-

dewed leaf fragment. The dishes containing the inoculated leaflets were then stored in the light at room temperatures, and readings of mildew severity were made after 7 to 9 days, and again on resistant leaflets two weeks after inoculation. Very little difficulty was caused by contamination with molds. To supplement such tests, potted plants in the greenhouse were also occasionally inoculated.

*Scale of Severity of Infection.*—The severity of the mildew was estimated on a scale of 0 to 4 as follows:

0 : No mycelium; spores germinate and may cause small necrotic spots by killing a few epidermal cells.

1 (trace) : Scant mycelium present; no spores; colonies few; necrotic spots present.

2 : Mycelium scant; a few spores formed; colonies few; necrotic spots present.

3 : Mycelium more abundant; spores formed rather generally on the mycelium; necrotic spots present.

4 : Mycelium and spores fairly abundant; very few necrotic spots.

Readings between 0 and 2 designate resistance; 3 and 4, susceptibility.

#### RELATIVE SUSCEPTIBILITY OF BEAN VARIETIES TO MILDEW

The relative severity of mildew on certain varieties of beans in the field as a result of natural infection and in the greenhouse as a result of inoculation of potted plants is compared in table 1 with the severity of mildew on inoculated detached leaflets of the same varieties. Inoculation of the detached leaflets is shown to be a more severe test than is afforded by field or greenhouse infection. In no instance did field or greenhouse infection occur on varieties showing less than a 2 reaction in the dish test. Numerous inoculation tests were made and the results in table 1 are typical.

The varieties Hungarian, Lady Washington, Pinto, Yellow, and Pink, were resistant; Frijole negros, Long Roman, and *Phaseolus vulgaris* 5003, intermediate; and Robust and Red Kidney susceptible. The Pink variety was slightly less resistant than the other four in that group, and in the seedling stage was much more susceptible than in later stages. Three other resistant varieties also showed a trace of infection in the seedling stage.

*Reaction of Parental Material—the Pinto, Long Roman, and Robust Varieties.*—As parental material for a study of the inheritance of mil-

dew resistance, the varieties Pinto, Long Roman, and Robust were selected. Pinto (4369) has always given a negative reaction in dish tests. In 1932, 1933, and 1935 no mildew developed on it in the field, but in 1934 a small amount developed owing to the presence of a new physiologic form of the mildew that year. Long Roman (4521) has been susceptible in dish tests, usually giving a reading of 3. In the field in 1932 no infection appeared before late in the fall when most varieties

TABLE 1

MILDEW SUSCEPTIBILITY OF CERTAIN VARIETIES AS INDICATED BY THE DISH TEST,  
BY NATURAL INFECTION IN THE FIELD, AND BY INOCULATION OF  
PLANTS IN THE GREENHOUSE, 1932-1933

	Variety†	Mildew readings*						Field notes, 1935†	
		Field 1932	Grown in green- house 1932-1933		Grown in field 1933				
			Plant‡ inocula- tion	Dish test, old	Field†	Dish test			
						Seedling	Old		
Resistant	Hungarian (4404).....	0	0	0	0	0	0	0	
	Lady Washington [84(213)82].....	0	0	0	0	t	0	0	
	Pinto (4369).....	0	0	0	0	0-t	0	0	
	Yellow (4429).....	0	0	0	0	t	0	0	
	Pink (4436).....	0	0	0-1	0	3-4	0-1	0	
Semi-resist- ant	Frijoles negros (5033) .....	0	m	2	0	3	2-3	m	
	Long Roman (4521).....	t	m	3	m	3-4	3	m	
	Phaseolus vulgaris (5003).....	t	m	3	m	3	3	m	
Suscep- tible	Robust (4458).....	3	m	4	m	4	3-4	m	
	Red Kidney (4764).....	4	m	4	m	4	4	m	

\* Field notes for 1934 are not included because a different strain of mildew was present which attacked the resistant varieties.

† It is merely indicated whether mildew developed (m) or not (0).

‡ Numbers given in this column are accession numbers of the California Experiment Station.

were harvested. In 1933 and 1935 a very small amount of infection was present, but in 1934 it was more severely infected. Robust (4458) from 1932 to 1935 showed a light to medium-heavy field infection. It is highly susceptible in the dishes, usually with a reading of 4.

#### MILDEW REACTION OF THE F<sub>1</sub>, F<sub>2</sub>, AND F<sub>3</sub> PROGENIES OF ROBUST X PINTO

Crosses between resistant and susceptible varieties were made in the greenhouse in the winter of 1932-33. The F<sub>1</sub> generation was grown in the field the following summer. F<sub>2</sub> generations have been grown both in the greenhouse and in the field. Only in the field has enough seed for conclusive F<sub>3</sub> tests been obtained. F<sub>3</sub> generations have been grown in the

greenhouse. For routine work the  $F_2$  and  $F_3$  were mostly planted in sterile soil in 6-inch pots, 6 seeds in each. Less damping-off was experienced in sterile soil than in nonsterile soil. To insure prompt germination, the seed was scarified. The temperature in the greenhouse has been about  $60^\circ$  to  $70^\circ$  F.

The  $F_1$  plants of the Robust  $\times$  Pinto cross and their parents were grown in the field. The mildew reaction was determined by the dish tests both in the seedling and in older stages. The  $F_1$  hybrids and their resistant Pinto parent were about equally resistant, as shown in table 2. Some additional  $F_1$  plants grown in the greenhouse were tested approxi-

TABLE 2  
MILDEW INFECTION ON ROBUST AND PINTO BEANS AND THEIR  $F_1$  AND  $F_2$  PROGENIES

Parent or cross	Number of plants with the mildew infection indicated						Total	$\frac{D}{E}$
	0	1	2	3	4			
Robust	0	0	0	0	3	15	18	....
Pinto	18	0	0	0	0	0	18	....
$F_1$	5	1	0	0	0	0	6	....
$F_2$	121	9	0	0	9	34	173	0.07

\*  $D$  Deviation from expected ratio number

E Probable error of the number of the population

mately every week during the first two months, and it was found that the mildew reaction of young plants was 0-1 and of older plants, almost always 0, like that of the resistant parent. The younger stages of Pinto showed somewhat more resistance than the younger stages of  $F_1$  hybrids, a fact which indicates that the complete pair of factors for resistance is more potent than a single one.

For the  $F_2$  generation, the progenies from two  $F_1$  plants were observed separately, but since the two sets presented no genetic differences they are treated together. One primary leaf from each  $F_2$  plant was tested for mildew susceptibility 7 to 10 days after the seedlings emerged and one leaf from each plant was tested when the plants were mature. The two tests were found to agree, so that in this cross the early test was reliable and susceptibility did not decrease with age. Furthermore the ratios of resistant to susceptible were the same in the plants tested twice as in those that had died before the second test was made. The results are included in table 2.

$F_1$  plants of Robust  $\times$  Pinto may give a reading of  $t$  at the age of two months. This approximates the age of the  $F_2$  plants at the second reading. For the resistant parent, Pinto, a reading of  $t$  was not obtained later

TABLE 3  
MILDEW READING OF F<sub>3</sub> PLANTS OF A ROBUST X PINTO CROSS

F <sub>3</sub> plant No.	Number of F <sub>3</sub> plants with readings						Ratio resistant susceptible	D — E	Families in F <sub>2</sub> group
	0	1	2	3	4				
4	24	9	..	..	..	..	33:0	....	12 homozygous resistant
6	27	7	1	..	..	..	35:0	....	
10	21	9	3	..	..	..	33:0	....	
13	31	3	..	..	..	..	34:0	....	
16	28	7	..	..	..	..	35:0	....	
18	26	8	..	..	..	..	34:0	....	
21	21	5	..	..	..	..	36:0	....	
25	25	8	..	..	..	..	33:0	....	
30	27	7	..	..	..	..	34:0	....	
36	30	5	..	..	..	..	35:0	....	
38	24	2	..	..	..	..	26:0	....	
41	29	4	..	..	..	..	33:0	....	
1	..	..	..	..	17	15	0:32	....	10 homozygous susceptible
8	..	..	..	..	10	24	0:34	....	
14	..	..	..	..	4	18	0:22	....	
19	..	..	..	..	..	17	0:17	....	
27	..	..	..	..	5	26	0:31	....	
33	..	..	..	..	3	14	0:17	....	
35	..	..	..	..	17	18	0:35	....	
42	..	..	..	..	7	25	0:32	....	
43	..	..	..	..	8	22	0:30	....	
45	..	..	..	..	4	23	0:27	....	
2	17	7	1	..	3	3	25:6	1.07	23 heterozygous resistant
3	9	18	1	..	4	3	28:7	1.01	
5	11	13	3	..	3	4	27:7	0.88	
7	9	12	3	..	7	4	24:11	1.30	
9	18	9	1	..	4	4	26:8	0.29	
11	16	8	..	..	5	..	29:5	2.06	
12	5	10	5	..	5	..	22:5	1.15	
15	13	13	..	..	6	4	26:10	0.57	
17	2	14	11	1	2	6	28:8	0.57	
20	13	10	2	..	5	4	27:7	0.88	
22	20	7	..	..	3	4	27:7	0.88	
23	17	10	..	..	9	..	27:9	0.00	
24	23	2	..	..	4	2	25:6	1.07	
26	14	9	3	..	5	5	25:10	0.72	
28	17	8	..	..	3	5	25:8	0.15	
29	19	6	..	..	9	..	25:9	0.29	
31	13	10	2	..	1	6	25:7	0.61	
32	14	7	..	..	4	4	21:8	0.48	
34	15	5	..	..	6	1	20:7	0.16	
37	9	10	4	1	5	7	24:12	1.71	
39	20	8	..	..	5	3	28:8	0.57	
40	14	6	..	..	6	3	20:9	1.11	
44	18	7	2	..	3	3	27:6	1.34	

than 16 days after planting; later readings were all 0. The  $F_2$  hybrids which in the second test showed a reading of  $t$  may thus be heterozygous rather than homozygous for resistance, while those that gave a reading of 0 may be either homozygous or heterozygous. Although a definite distinction between plants homozygous and heterozygous for resistance was not established, they are both in the resistant group, which by lack of plants in the intermediate classes 1 and 2 is distinctly separated from the susceptible group. The ratio 130:43 fits almost perfectly a 3:1 ratio and indicates that resistance to mildew in Pinto is due to a single dominant Mendelian factor.<sup>10</sup>

From each of 45  $F_2$  plants (the progeny of one  $F_1$  plant) grown in the field in 1934, 36 seeds were planted in the greenhouse the following winter. The mildew reaction of the  $F_2$  parents was unknown. The mildew reaction of each plant of the 45  $F_3$  progenies was determined by dish tests in the young stage. The results, including the ratio of resistant to susceptible plants in each family, are given in table 3. The  $F_3$  families are grouped on the basis of the readings, and it may be seen that the 45 parental  $F_2$  plants may be classified as follows: homozygous resistant, 12; heterozygous resistant, 23; homozygous susceptible, 10. This is a ratio of 35 resistant to 10 susceptible. The  $\frac{D}{E}$  is 0.64. This approximates a 1:2:1 ratio or a ratio of 3 resistant : 1 susceptible, as was found in the tests with the  $F_2$  generation, and establishes that the resistance in Pinto is due to a main single Mendelian factor pair.

There is a rather wide variation in the readings of the 23 heterozygous  $F_3$  families. For instance family 17 has the average of its 28 resistant readings between  $t$  and 1 with two plants in the 0 class and one in the 2 class, while family 22 has 20 readings of 0 and 7 of  $t$ , with none in classes 1 and 2. This indicates that there are factors or combinations of factors present which modify the resistance. Although some of the heterozygous  $F_3$  families have more plants with intermediate readings than did the  $F_2$  generation as shown in table 2, there is nevertheless a distinct difference between the resistant and susceptible plants.

#### MILDEW REACTION OF THE $F_1$ , $F_2$ , AND $F_3$ PROGENIES OF LONG ROMAN $\times$ PINTO

It will be recalled that Pinto is resistant and Long Roman is somewhat resistant in the field but shows susceptibility in the dish tests. The seven  $F_1$  plants were grown in the field, and when tested in the dishes all were resistant to the mildew and gave a reading of 0, like the resistant parent Pinto (table 4).

An  $F_2$  generation was grown in the greenhouse. Tests made when the plants were young showed more variation in mildew resistance than did tests made when the plants were older, and a few plants changed from susceptible when young to resistant when older. The readings given in table 4 represent the average of 7 to 10 individual leaves taken from each plant at different times and tested by the dish method. The constancy of the later readings and the rather distinct separation of resistant and

TABLE 4  
MILDEW INFECTION ON LONG ROMAN AND PINTO BEANS AND THEIR  
 $F_1$  AND  $F_2$  PROGENIES

Parent or cross	Number of plants with the mildew infection indicated						Total	$\frac{D^*}{E}$
	0	t	1	2	3	4		
<i>Grown in greenhouse</i>								
Long Roman.....	0	0	0	0	9	0	9	....
Pinto.....	9	0	0	0	0	0	9	....
$F_1$ .....	45	29	9	0	8	10	101	2.47
<i>Grown in field</i>								
Long Roman.....	0	0	0	0	13	0	13	....
Pinto.....	13	0	0	0	0	0	13	....
$F_1$ .....	7	0	0	0	0	0	7	....
$F_2$ .....	53	15	13	4	12	0	106	1.83

\* D Deviation from expected ratio number

E Probable error of the number of the population

susceptible groups by the absence of class 2 as shown in table 4 indicate that the  $F_2$  generation should give a reliable picture of the inheritance of resistance. Although there are a larger number of readings of t and 1 than in the Robust  $\times$  Pinto  $F_2$  hybrids, the results, like those of the previous cross, indicate the presence of a main factor pair for resistance in the Pinto variety.

$F_2$  plants from seed from the same  $F_1$  plant used for those grown in the greenhouse were also grown in the field in 1934. Seedlings were tested in the dishes for mildew resistance within 4 days after emergence and a second test was made when the plants began to bloom. The readings on the older plants are given in table 4. They give about the same fit to a 3:1 ratio of susceptible to resistant as the greenhouse series. A few plants changed from susceptible in the seedling stage to resistant when older. There are 4 plants with a reading of 2, and class 3 is relatively larger than in the greenhouse series. A similar difference between field and greenhouse-grown plants has been observed in other trials.

The  $F_2$  generation was grown from seed from 47 resistant and 10 susceptible  $F_2$  plants grown in the field in 1934, including the 4 plants with

TABLE 5

MILDEW READINGS OF  $F_2$  PLANTS AND THEIR  $F_2$  PROGENY FROM A LONG  
ROMAN X PINTO CROSS

$F_2$		$F_2$						Ratio resistant susceptible	$D^*$ $E$
Mil- dew read- ing	Plant No.	0	1	2	3	4			
0	1	28	6	0	1	0	0	35:0	...
	2	24	8	2	0	0	0	34:0	...
	3	25	3	0	0	0	0	28:0	...
	4	19	8	2	1	0	0	30:0	...
	5	34	1	0	0	0	0	35:0	...
	6	21	7	2	3	0	0	33:0	...
	7	13	4	3	7	0	0	27:0	...
	8	33	1	0	0	0	0	34:0	...
	9	27	2	0	0	0	0	29:0	...
	10	31	2	0	0	0	0	33:0	...
	11	14	1	0	0	4	0	15:4	0.59
	12	26	0	0	0	4	3	26:7	0.74
	13	22	6	1	0	4	1	29:5	2.06
	14	40	2	0	0	8	5	42:13	0.35
	15	19	1	2	0	6	0	22:6	0.65
	16	28	2	0	0	6	0	30:6	1.71
	17	26	1	0	0	6	2	27:8	0.43
	18	23	6	0	0	7	0	29:7	1.14
	19	24	1	0	1	7	0	26:7	0.74
	20	20	0	0	0	5	0	20:5	0.86
	21	17	2	0	0	5	4	19:9	1.29
	22	19	6	0	0	8	2	25:10	0.72
1	23	39	0	0	0	0	0	39:0	...
	24	39	0	0	0	0	0	39:0	...
	25	19	6	3	0	0	0	38:0	...
	26	25	10	0	1	0	0	36:0	...
	27	27	8	1	0	0	0	36:0	...
	28	24	3	0	2	1†	0	29:1	...
	29	18	5	1	0	7	2	24:9	0.45
	30	32	0	0	0	7	0	32:7	1.51
	31	30	1	0	0	5	4	31:9	0.54
	32	22	1	0	0	7	1	23:8	0.15
	33	19	8	0	0	8	1	27:9	0.00
	34	30	8	0	0	9	1	40:10	1.21
	35	25	1	0	0	7	0	26:7	0.74
	36	22	3	0	0	5	1	25:6	1.07
2	37	19	1	1	0	2	8	21:10	1.38
	38	18	2	1	1	4	10	22:14	2.86
	39	20	10	0	1	4	1	21:4	1.54
	40	20	3	0	0	4	1	23:5	1.29
	41	36	1	0	1	7	1	38:9	1.38
	42	28	4	2	1	14	0	35:14	0.86
	43	19	2	0	0	6	5	21:11	1.82
3	44	16	9	1	3	7	12	39:19	2.03
	45	16	7	0	0	4	4	23:8	0.15
	46	14	8	0	0	5	6	22:6	0.65
	47	14	5	2	0	5	4	21:11	1.82
4	48	0	0	0	0	5	10	0:15	...
	49	0	0	0	0	10	12	0:22	...
	50	0	0	0	0	4	18	0:22	...
	51	0	0	0	0	15	8	0:23	...
	52	0	0	0	0	11	5	0:16	...
	53	0	0	0	0	10	15	0:25	...
	54	0	0	0	0	12	12	0:24	...
	55	0	0	0	0	12	23	0:35	...
	56	0	0	0	0	2	16	0:18	...
	57	0	0	0	0	7	15	0:22	...

\*  $D$  Deviation from expected ratio number\*  $E$  Probable error of the number of the population

† Thought to be the result of a mixture.

a mildew reading of 2 (table 4). The  $F_2$  plants were the progenies of one  $F_1$  plant. The populations of the 47  $F_3$  families from resistant  $F_2$  plants range from 19 to 58 with only 9 below 30. The susceptible  $F_2$  plants (mildew reading of 3 or 4) yielded a comparatively small amount of seed owing to the injury from mildew, and the population of their  $F_3$  families ranges from 15 to 36.

Of the 47 resistant  $F_2$  plants (readings 0,  $t$ , 1, and 2) tested, 16 proved to be homozygous and 31 heterozygous for resistance, a result which is very close to the 1:2 ratio expected from a random sample. The 31 heterozygous  $F_2$  plants segregated in the  $F_3$ , in accordance with the single-factor hypothesis. The susceptible  $F_2$  plants (readings 3 and 4) gave only susceptible progeny. The resistance observed in Long Roman has not interfered with the expression of the Pinto resistance in this test.

The  $F_2$  readings give an indication as to the homozygosity or heterozygosity of the plants in that  $F_2$  mildew readings of 1 and 2 all represented heterozygous plants, while readings of 0 and  $t$  represented both homozygous and heterozygous plants in about equal numbers.

#### SUMMARY

For use in determining the susceptibility of beans (*Phaseolus vulgaris* L.) to powdery mildew (*Erysiphe polygoni* D.C.), the mildew was grown in petri dishes on detached bean leaflets supported on cotton soaked in a 10 per cent sucrose solution. Use of the dish-culture method permitted the continuous use of one physiological race of the mildew and offered numerous other advantages.

The susceptibility of different varieties and individual plants was determined by inoculating detached leaves. The results were in most cases found to be in close agreement with field and greenhouse infection. In no case was infection less severe in the dishes. Mildew readings were made on a scale of 0 to 4.

The varieties Hungarian, Lady Washington, Pinto, and Pink were found to be resistant, and Robust and Red Kidney were susceptible, as tested in the dishes and in field and greenhouse. Frijole negros was resistant in the field and greenhouse, but semiresistant in the dishes. Long Roman was semiresistant in the field and greenhouse and susceptible in the dishes.

The  $F_1$ ,  $F_2$ , and  $F_3$  progenies of a cross between the susceptible Robust and the resistant Pinto were tested by the dish method. Of the 45  $F_2$  plants tested, 12 proved to be homozygous resistant, 23 heterozygous resistant, and 10 homozygous susceptible.

The  $F_1$ ,  $F_2$ , and  $F_3$  progenies of a cross between the semiresistant

Long Roman and the resistant Pinto were similarly tested and of the 47 resistant  $F_2$  plants tested, 16 proved to be homozygous and 31 heterozygous. Readings of 0 and  $t$  in the  $F_2$  plants indicated homozygosity or heterozygosity for resistance; 1 and 2, heterozygosity only; and 3 and 4, homozygosity for susceptibility.

In the crosses with Robust and Long Roman, the Pinto is seen to have a single Mendelian factor pair for resistance to the strain of powdery mildew used.

Plants were often somewhat more susceptible in the seedling stage than later.

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## **SPOTTED WILT OF THE SWEET PEA**

**W. C. SNYDER AND H. REX THOMAS**



# SPOTTED WILT OF THE SWEET PEA<sup>1</sup>

W. C. SNYDER<sup>2</sup> AND H. REX THOMAS<sup>3</sup>

## INTRODUCTION

IN THE COASTAL COUNTIES of California the sweet pea, *Lathyrus odoratus* L., is frequently afflicted with a streak disease. The cause of the disease is sometimes obscured by viruses of the mosaic type or by resemblance to the description of bacterial streak of sweet pea.<sup>(m)</sup><sup>1</sup> It has, however, been proved to be a virus identical with that responsible for the spotted wilt disease of tomato. Recently<sup>(m)</sup> this virus has been reported as the cause of a streak disease of the garden pea, *Pisum sativum* L.

## SYMPTOMS

A characteristic symptom of the disease on sweet pea is a necrotic streaking of stems and petioles. The streaks are reddish brown to dull purple in color and in the advanced stage are conspicuous. They may run the entire length of the stem, parallel to the long axis, being found on analysis to consist of disconnected short streaks or a continuous long one. They have been seen to develop both above and below the point of virus inception.

Leaves and shoots may turn yellow and die. Early symptoms on the foliage appear as spots, usually 5 to 15 mm long, more or less circular or oval in shape, yellowish at first, with diffuse margins. Later the spots become somewhat brownish and form a pattern typical of the virus on other hosts. From these spots are developed yellowish sectors or zones, ordinarily one or only a few on each leaf. The spotting of the foliage is fairly definite in early stages, although not striking; but the general yellowing which may follow becomes a conspicuous symptom. Symptoms of the disease are shown in figure 1, A and D.

Blossoms on infected plants have been seen to develop a circular pattern in the pigment and the virus has been recovered from such material. Again, blighting of the whole shoot may occur prior to blossom formation.

Variability in the expression of symptoms has been observed. Plants infected early may yellow and die without showing other symptoms. In other cases the virus may be limited to local lesions instead of becoming

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\* Superscript numbers in parentheses refer to Literature Cited at end of this paper.



Fig. 1.—Symptoms of the spotted-wilt virus: *A*, Yellowish foliage spots on sweet pea. *B*, Brown foliage spots and blighting on garden pea. *C*, Pitted necrosis of pod of garden pea. Ovules failed to develop in this pod. *D*, Dark reddish brown streak on stem of sweet pea. *E*, Circular pattern on pod of garden pea ( $\times 2$ ). .

systemic. This is frequently the case in artificial inoculations wherein lesions remain confined to the zone immediately above and below the inoculated leaves. Such infection does not result in the severe stunting and destructiveness obtained with systemic infections unless, as sometimes happens, the stem is so weakened that it breaks at the point of inoculation.

### INOCULATION EXPERIMENTS

The diseased sweet-pea plants first used successfully as inoculum in the artificial transmission of the virus and in its determination were obtained in November. They were taken from a severe infestation of streak in a greenhouse devoted to sweet-pea culture at San Pablo, where spotted wilt is prevalent. The plants, only a few inches aboveground when infected, showed stem streaking, spotting of the foliage, general yellowing, and blighting of the shoots. They were free from mosaics. In some cases symptoms were confined to the blighted shoot, since subsequent shoots developed on the same root seemed to be normal for the time being. In other instances the virus became systemic, and where such plants survived to produce bloom, they sometimes bore flowers showing pigment patterns in the petals, as described above.

Expressed juice from the naturally infected sweet peas was inoculated by means of the carborundum rubbing method<sup>10</sup> into healthy sweet peas and test plants for the spotted wilt virus. In four trials with fresh collections of the diseased sweet peas, the virus was successfully transmitted into *Nicotiana glutinosa* L., *N. tabacum* L., *Datura stramonium* L., and *Lycopersicum esculentum* Mill. Local lesions obtained on *N. glutinosa* and *N. tabacum*, as well as systemic infections induced in these and in *D. stramonium* and *L. esculentum* were all typical for the spotted wilt virus.

The sweet-pea plants which became infected resembled the original material, and the virus was recovered from them in turn on the test plants. Although transmission of the virus from sweet pea to the other hosts used in these trials was obtained fairly readily, the percentage of sweet-pea plants which became infected was low, varying from 0 to 60 per cent. This is in line with experience in other instances where the spotted wilt virus has been concerned. The incubation period varied usually from 14 to 30 days.

These results indicate that the spotted wilt virus of tomato was present in the diseased sweet peas, and that it could be recovered on other host plants by the mechanical method of transmission used.

By reversing the procedure of inoculation, healthy sweet peas (Red Boy variety) were inoculated from a tomato plant containing the spotted

ing a vector of the spotted wilt virus, may be used to isolate the mosaic virus where mosaic and spotted wilt are present in the same plant. In mechanical inoculations with expressed juice the mosaics are usually transmitted in a much higher percentage of cases than spotted wilt. As a result only the mosaic virus may be recovered when the juice from a plant containing both viruses is applied mechanically to a common host. Other streaks of pea, however, may be more readily transmitted by mechanical inoculation.

Control of streak would seem to lie in the isolation of sweet pea plantings from crops susceptible to spotted wilt and from infested districts, or in the protection of plants from migrations of infective thrips.

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## ORNAMENTAL FLOWERING PLANTS EXPERIMENTALLY INFECTED WITH CURLY TOP<sup>1</sup>

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### INTRODUCTION

SEVERAL INVESTIGATORS have recorded the experimental transmission of virus diseases of plants to a large number of species in different genera of many families. A review of the literature on the host range of certain virus diseases shows an extensive host range as determined by experimental infection, but the reported natural host range is often limited, as illustrated in table 1.

The natural infection of ornamental flowering plants with curly top has already been reported in a previous paper.<sup>(2)</sup> A list of the plants experimentally infected, but without details of the experiments or description of the symptoms, was published in the *Plant Disease Reporter*.<sup>(3)</sup> The details and results of the experiments performed to experimentally infect ornamental flowering plants with curly top are given in the present paper. No intensive investigations have been conducted to determine which plants are resistant or immune to curly top; only plants experimentally infected with the disease are reported in this paper. The symptoms on the susceptible host plants are briefly described. The longevity of the last living male and female beet leafhopper, *Eutettix tenellus* (Baker), was ascertained on inoculated plants and a record was kept of all plants on which the leafhopper completed its life cycle.

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<sup>4</sup> Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

## METHODS OF EXPERIMENTALLY INFECTING PLANTS WITH CURLY TOP

The methods used in the transmission of curly top to ornamental flowering plants were somewhat similar to those described in the experimental infection of economic plants and weeds reported in an earlier contribution.<sup>(23)</sup> The ornamental flowering plants were grown from seeds in the

TABLE 1  
HOST RANGES OF SOME PLANT VIRUS DISEASES AS DETERMINED BY NATURAL  
AND EXPERIMENTAL INFECTION

Virus disease	Authority*	Locality	Plants experimentally infected			Plants naturally infected		
			Species	Genera	Families	Species	Genera	Families
Sugar beet curly top	{ Severin and co- workers (7, 14, 15, 17, 18, 19, 20, 25, 26, 27, 28) Carsner (6)	California	194	122	40	75	48	18
		California	14	13	8	..	..	..
Aster yellows	{ Kunkel (11, 12) Severin (21, 24, 26, (27)	New York	170	150	38	9	9	4
		California	7	5	2	14	13	6
Southern celery mosaic	Wellman (33, 34)	Florida	81	59	23	42	35	17
Tobacco ring- spot	Wingard (35, 36)	Virginia	62	38	17	11	9	3
Tomato spotted wilt	{ Bald and Samuel (5) Smith (29, 30, 31) Gardner and co- workers (8, 9, 32)	Australia	35	15	4	4	4	4
		England	28	13	4	4	4	4
		California	30	24	19	31	31	17
Tobacco mosaic	Grant (10)	Wisconsin	29	24	14	..	..	..
Tomato bunchy top	McClean (13)	South Africa	15	8	2	..	..	..

\* Numbers in parentheses refer to numbers in list of "Literature Cited" at the end of this paper.

greenhouse and were fumigated with Nico-Fume tobacco-paper insecticide prior to using. Each plant was enclosed in a cage and inoculated with curly top by either infective male or female beet leafhoppers which had completed their nymphal stages on beets in an advanced stage of the disease. The number of insects used varied from 5 to 20, according to the size of the plants. When the longevity of the adults was short, presumably because of unfavorable food, the plants were repeatedly inoculated by different lots of infective insects.

The plants were exposed to the infective leafhoppers until symptoms of curly top developed, which usually required about two weeks; if no symptoms appeared, the insects were kept on the plants for a period of 6 weeks. After inoculation the cage containing the infective specimens was removed, and care was taken to see that the plant was free from all insects.

Healthy plants of each species or variety were exposed to noninfective beet leafhoppers as a check, and as a further check, healthy plants were grown in cages free from insects.

No species or variety of ornamental flowering plant inoculated with curly top was considered to be infected with the disease on the basis of symptoms, unless the virus was recovered by previously noninfective male leafhoppers or nymphs and transferred to sugar beets. From 2 to 10 plants of each species or variety were inoculated, as indicated in table 2. Small plants were ordinarily used, but sometimes plants susceptible to curly top would die before the virus could be transferred to sugar beets, and hence larger plants were inoculated.

In one method of recovering the virus, the inoculated plants were exposed to previously noninfective male leafhoppers enclosed in a cage for a period of 2 or 3 days, or longer if the food material was favorable. (In order to avoid egg deposition, females were not used in this type of recovery tests.) The insects were then transferred from the inoculated plant to two healthy beet seedlings, each enclosed in a cage. Two beet seedlings were used in each test because occasionally a seedling is killed by damping-off. The leafhoppers were confined in cages enclosing the beet seedlings for a period of 5 to 7 days, and then the cages containing the leafhoppers were removed.

In another method of recovering the virus, nymphs which hatched from eggs deposited in favorable food plants by infective females used to inoculate the plants, were transferred to beet seedlings. It has been established<sup>(16, 20)</sup> that nymphs which hatch from eggs deposited by infective females are not able to transmit curly top unless the nymphs have previously fed on a diseased plant.

The inoculated beets were fumigated with Nico-Fume tobacco-paper insecticide and were kept within insect-proof cages for a period of 3 months, if symptoms of curly top did not develop within the usual period of 1 to 2 weeks. If the beets developed curly top, it is evident that the inoculated ornamental flowering plant had been infected with the disease.

TABLE 2  
List of Ornamental Flowering Plants Experimentally Infected with Curly Top, Longevity,  
and Life Cycle\* of Beet Leafhopper†

Family	Common	Name of plant	Scientific	Seasons duration	Number of plants	Longevity: days	
						Inoculated	Virus recovered
Moraceae.....	Japanese hop.....	<i>Humulus japonicus</i> Sieb. and Zucc.	<i>Kochia trichophylla</i> Stapf.	Perennial grown as annual	3	3	16
Chenopodiaceae.....	Common summer-cypress.....			Annual.....	3	3	14-18
	Joseph's-coat.....			Annual.....	3	3	C*
Amaranthaceae.....	Love-lies-bleeding.....	<i>Amaranthus viridis</i> L.	<i>Amaranthus viridis</i> L.	Annual.....	3	3	C
	Feather cockscomb.....	<i>Amaranthus caudatus</i> L.	<i>Amaranthus caudatus</i> L.	Annual.....	3	3	C
Nyctaginaceae.....	Common globe-amaranth.....	<i>Celosia argentea</i> L.	<i>Celosia argentea</i> L.	Annual.....	3	3	C
Portulacaceae.....	Common four-o'clock.....	<i>Gomphrena globosa</i> L.	<i>Gomphrena globosa</i> L.	Annual.....	8	8	C
	Common portulaca.....	<i>Mirabilis jalapa</i> L.	<i>Mirabilis jalapa</i> L.	Perennial grown as annual	6	6	C
	Common rock-purslane.....	<i>Portulaca grandiflora</i> Hook.	<i>Portulaca grandiflora</i> Hook.	Annual.....	7	5	C
	Sweet william.....	<i>Calandrinia grandiflora</i> Lindl.	<i>Calandrinia grandiflora</i> Lindl.	Annual.....	2	2	C
	Chinese pink.....	<i>Dianthus barbatus</i> L.	<i>Dianthus chinensis</i> L.	Perennial.....	6	6	C
	Heddewig's pink.....	<i>Dianthus chinensis</i> L. var. <i>hedwigii</i> Regel.	<i>Dianthus chinensis</i> L. var. <i>hedwigii</i> Regel.	Perennial grown as annual	5	3	C
Caryophyllaceae.....	Grass pink.....	<i>Dianthus plumarius</i> L.	<i>Dianthus plumarius</i> L.	Perennial.....	7	6	C
	Carnation.....	<i>Dianthus caryophyllus</i> L.	<i>Dianthus caryophyllus</i> L.	Perennial.....	7	3	3-12
	Baby's breath.....	<i>Gypsophila paniculata</i> L.	<i>Gypsophila paniculata</i> L.	Perennial.....	5	3	C
	Drooping catchfly.....	<i>Silene pendula</i> L.	<i>Silene pendula</i> L.	Annual.....	5	4	C
	Mallows cross.....	<i>Lachnis chalcidonica</i> L.	<i>Lachnus chalcidonica</i> L.	Perennial.....	4	3	C
	Hedge campion.....	<i>Lachnis hedgesii</i> Lem.	<i>Lachnus hedgesii</i> Lem.	Perennial.....	6	5	C
	Poppy anemone.....	<i>Anemone coronaria</i> L.	<i>Anemone coronaria</i> L.	Perennial.....	5	5	48
Ranunculaceae.....	Love-in-a-mist.....	<i>Nigella damascena</i> L.	<i>Nigella damascena</i> L.	Annual.....	6	6	C
	Orange lar-asper.....	<i>Delphinium nudicaule</i> Torr. & Gray.	<i>Delphinium nudicaule</i> Torr. & Gray.	Perennial.....	2	2	C
	Iceland poppy.....	<i>Papaver nudicaule</i> L.	<i>Papaver nudicaule</i> L.	Perennial.....	7	6	16-36
	Oriental poppy.....			Perennial.....	7	3	7-12
	Salmon Queen variety, Oriental poppy.....	<i>Papaver orientale</i> L.	<i>Papaver orientale</i> L.	Perennial.....	7	5	6-25
	Oriental poppy hybrid.....			Perennial.....	7	2	20-30
							54

\* C indicates that the beet leafhopper completed its life cycle on host plant.

† The common names of the ornamental flowering plants were obtained from Standardized Plant Names (1), or if the common names were not given in this publication, then from Bailey (2, 3) or L. H. Bailey and E. Z. Bailey (4).

TABLE 2—(Continued)

Family	Common	Name of plant	Scientific	Seasons duration		Number of plants	Virus recovered	Males	Females	Longevity, days
				Inoculated	.....					
Capparidaceae	Spiderflower	<i>Cleome spinosa</i> Jacq.	.....	Annual	.....	6	5	..	C*	..
	Annual stock	<i>Matioliola incana</i> R. Br. var. <i>annua</i> Voss	.....	Annual	.....	4	3	..	C	..
Cruciferae	Dames rocket	<i>Hesperis matronalis</i> L.	Biennial or perennial	.....	.....	3	3	..	C	..
	Honeywort	<i>Lunaria annua</i> L.	Annual or biennial	.....	.....	5	5	..	C	..
Rubiaceae	Purple candy-tuft	<i>Iberis umbellata</i> L.	Annual	.....	.....	6	3	..	C	..
	Common mignonette	<i>Resseliella odorata</i> L.	Annual	.....	.....	6	6	..	C	..
Geraniaceae	Fish geranium	<i>Felargonium horodorum</i> Bailey	Perennial	.....	.....	3	2	5-16	8-16	..
Tropaeolaceae	Canary nasturtium	<i>Tropaeolum peruvianum</i> L.	Annual	.....	.....	2	2	..	C	..
Euphorbiaceae	Show-on-the-mountain	<i>Euphorbia marginata</i> Pursh	Annual	.....	.....	5	3	4-8	11-18	..
Sapindaceae	Balloongrass	<i>Cardiospermum halicacabum</i> L.	Perennial grown as annual	.....	.....	2	2	..	C	..
Malvaceae	Herbtreemallow	<i>Desmodium trinervia</i> L.	Annual	.....	.....	2	2	..	C	..
	Tufted pansy	<i>Viola cornuta</i> L.	Perennial	.....	.....	4	4	..	C	..
Violaceae	Clarkia	<i>Clarkia elegans</i> Dougl.	Annual	.....	.....	4	4	..	C	..
Onagraceae	Blue laceflower	<i>Trichomea caerulea</i> R. Graham	Annual	.....	.....	5	4	24	..	..
Umbelliferae	Top primrose	<i>Primula obconica</i> Hance	Perennial	.....	.....	1	1	..	C	..
Primulaceae	Cowslip primrose	<i>Primula satulata</i> Komar.	Perennial	.....	.....	2	2	..	C	..
	Oxlip primrose	<i>Primula verna</i> L.	Perennial	.....	.....	3	3	..	C	..
Plumbaginaceae	Notched sea-lavender	<i>Primalium elatior</i> Hill.	Perennial	.....	.....	4	4	..	C	..
Apoynaceae	Madagascar periwinkle	<i>Limonium sinuatum</i> Mill.	Biennial or perennial	.....	.....	6	4	..	C	..
Convolvulaceae	Crimson star-glory	<i>Vinca rosea</i> L.	Perennial grown as annual	.....	.....	6	5	30-75	80-92	..
	Brasillian morning-glory	<i>Quamoclit lobata</i> House	Perennial	.....	.....	4	1	1	5-20	11-24
Polemoniaceae	Drammond phlox	<i>Polemonium drummondii</i> Hook.	Annual	.....	.....	3	3	20-40	43	..
	Spotted bellflower	<i>Globaea scandens</i> Cav.	Perennial grown as annual	.....	.....	2	2	6-8	12-26	..
Hydrophyllaceae	Alkanet	<i>Nemophila maculata</i> Benth.	Perennial	.....	.....	4	4	..	C	..
Boraginaceae	Chinese forget-me-not	<i>Anchusa azurea</i> Mill.	Perennial	.....	.....	8	6	4-20	12-31	..
	True forget-me-not	<i>Cynoglossum amabile</i> Stapf and Drum.	Perennial	.....	.....	5	5	..	C	..
	Common heliotrope	<i>Myosotis scorpioides</i> L.	Perennial	.....	.....	4	4	..	C	..
		<i>Heliotropium peruvianum</i> L.	Perennial	.....	.....	6	3	..	C	..

\* C indicates that the beet leafhopper completed its life cycle on host plant.

Table 2 continued on page 868.

TABLE 2—(Continued)

Family	Name of plant		Seasons duration	Number of plants		Longevity, days	
	Common	Scientific		Inoculated	Virus recovered	Males	Females
Labiatae.	Scarlet sage..... Jasmine tobacco.....	<i>Salvia splendens</i> Ker-Gawl. <i>Nicotiana alata</i> Link & Otto var. <i>grandiflora</i> Comes.....	Perennial grown as annual Perennial grown as annual	2 4	2 3	3-8 6-7	12-15 20-30
Solanaceae.	Common petunia (Rose Morn variety)..... Scalloped salpiglossis..... Weston butterflyflower.....	<i>Petunia hybrida</i> Vilm. <i>Salpiglossis sinuata</i> Rids and Pav. <i>Browallia speciosa</i> Hook. <i>Schizanthus tricolorensis</i> Low.....	Perennial grown as annual Annual..... Annual..... Annual or biennial.....	2 4 4 3	2 2 4 3	1-2 5-8 4-6 ..	1-3 12-30 6-11 C*
Sorophulariacae.	Yellow foxglove..... Kenilworth ivy.....	<i>Digitalis ambigua</i> Murr. <i>Cymbalaria muralis</i> Gaertn., Mey. & Scherb.	Biennial or perennial.....	7	3	..	C
Acanthaceae.	Pouched nemesis..... Golden monkeyflower..... Black-eyed clock vine.....	<i>Nemastia strumosa</i> Benth. <i>Mimulus luteus</i> L. <i>Thunbergia alata</i> Bojer	Perennial..... Annual..... Perennial.....	3 2 2	2 2 2	3-20	25
Cucurbitaceae.	Mock-orange gourd..... Net-sack gourd..... Turks-turban gourd..... Suakwa tovalogourd..... Calabash gourd..... Dipper-shaped gourd..... Hercules-club gourd..... Powder-horn gourd..... Spoon gourd..... Snakegourd.....	<i>Cucurbita pepo</i> L. var. <i>ovifera</i> Bailey <i>Cucurbita pepo</i> L. var. <i>ovifera</i> Bailey <i>Luffa cylindrica</i> Roem. <i>Lagenaria leucantha</i> Rusby <i>Lagenaria leucantha</i> Rusby..... <i>Lagenaria leucantha</i> Rusby..... <i>Lagenaria leucantha</i> Rusby..... <i>Lagenaria leucantha</i> Rusby..... <i>Treloanthus angustifolia</i> L.	Perennial grown as annual Annual..... Annual..... Annual..... Annual..... Annual..... Annual..... Annual..... Annual..... Annual.....	3 6 2 2 2 5 3 2 2 3	3 5 2 2 2 3 3 2 2 3	3-16 3-11 .. 7-21 .. 3-11 3-11 4-11 6-17 4-8	6-51 10-49 C 38 C 3-28 7-26 24 8-9 19
Lobeliaceae.	White-eye lobelia..... Cardinalflower.....	<i>Lobelia erinus</i> L. var. <i>speciosa</i> ..... <i>Lobelia cardinalis</i> L.	Annual..... Perennial.....	10 4	6 4	4-14 8-16	6-30 17-36

\* C indicates that the beet leafhopper completed its life cycle on host plant.

TABLE 2—(Concluded)

Family	Name of plant		Seasons duration	Number of plants		Longevity, days
	Common	Scientific		Inoculated	Virus recovered	
	Crownbeauty.....	<i>Chrysanthemum coronarium</i> L.	Annual.....	2	2	1
	Feverfew.....	<i>Chrysanthemum parthenium</i> Pers.	Perennial.....	10	5	C*
	Marguerite.....	<i>Chrysanthemum frutescens</i> L.	Perennial.....	2	2	C
	Senileless false-camomile.....	<i>Matricaria inodora</i> L.	Annual.....	6	4	C
	Cucumber sunflower.....	<i>Helianthus debilis</i> Nutt.	Annual.....	7	4	50
	Golden thistle sunflower.....	<i>Helianthus decapetalus</i> L. var. <i>multi-florus</i> Hort.	Perennial.....	6	6	C
	Common sunnia.....	<i>Zinnia elegans</i> Jacq.	Annual.....	25	25	2-9
	Orange sunnia.....	<i>Zinnia elegans</i> Regel	Annual.....	2	2	3-4
	Common cosmos.....	<i>Cosmos bipinnatus</i> Cav.	Annual.....	2	2	18
	Giant-flowering cosmos.....	<i>Cosmos bipinnatus</i> Cav.	Annual.....	7	4	C
	Crested cosmos.....	<i>Cosmos bipinnatus</i> Hort.	Annual.....	6	4	C
	Calliopsis.....	<i>Coreopsis tinctoria</i> Nutt.	Annual.....	10	5	C
	English daisy.....	<i>Bellis perennis</i> L.	Perennial.....	3	3	C
	Swan-river-daisy.....	<i>Brachycome Iberidifolia</i> Benth.	Annual.....	3	3	C
	Bushy acajita.....	<i>Arcotia strobifolia</i> Berg.	Annual.....	3	3	C
	French marigold.....	<i>Tagetes patula</i> L.	Annual.....	10	6	C
	Astec marigold (African marigold).....	<i>Tagetes erecta</i> L.	Annual.....	6	3	C
	Winter cape-marigold.....	<i>Dimorphotheca aurantiaca</i> DC.	Perennial.....	3	3	C
	Pot-marigold.....	<i>Calendula officinalis</i> L.	Annual.....	9	6	C
	Rose everlasting.....	<i>Hedypnois rosea</i> Benth.	Annual.....	4	4	C
	Strawflower.....	<i>Helichrysum bracteatum</i> Andr.	Annual.....	6	6	C
	Sweet-sultan.....	<i>Centauraea moschata</i> L.	Annual.....	10	7	C
	Basketflower.....	<i>Centauraea mericana</i> Nutt.	Annual.....	5	3	C
	Cornflower.....	<i>Centauraea cyanus</i> L.	Annual.....	6	6	C

\* C indicates that the beet leafhopper completed its life cycle on host plant.

## HOST RANGE OF CURLY TOP AMONG ORNAMENTAL FLOWERING PLANTS

The number of ornamental flowering plants inoculated with curly top and the number of infected plants from which the virus was recovered by previously noninfective beet leafhoppers and transferred to beet seedlings is shown in table 2. The host range of curly top among ornamental flowering plants includes 92 species in 73 genera belonging to 33 families.

Many species of ornamental flowering plants repeatedly inoculated by different lots of infective beet leafhoppers showed no foliage symptoms and the virus was not recovered from such plants. These plants apparently are either immune or resistant to curly top and are not listed in table 2.

### SYMPTOMS OF CURLY TOP

Environmental conditions are important factors influencing the expression of curly-top symptoms. High humidity and high temperatures with a range of 70° to 110° F were maintained in the greenhouse during the summer months. Certain species of plants which displayed no outstanding symptoms of curly top might have developed them under different humidity and temperatures.

Ornamental flowering plants infected with curly top show a variation in symptoms. Many plants develop some of the symptoms developed by the sugar beet as described in a previous paper.<sup>(2)</sup> Other infected plants such as Madagascar periwinkle (*Vinca rosea*), common heliotrope (*Heliotropium peruvianum*), and purple candytuft (*Iberis umbellata*) did not develop symptoms. Either such species are symptomless carriers of the disease or the symptoms are entirely masked under greenhouse conditions.

The effect of the disease on the flowers of many species was not studied in detail. Young plants infected with curly top frequently produce no flowers as they mature. Older plants infected before blooming often develop dwarfed, malformed, and fewer flowers than healthy plants. Some species of infected plants produce normal flowers.

A brief description of symptoms on the individual species follows. These symptoms are described in many instances from young infected plants. Such symptoms are often more pronounced than those shown by plants infected at a later stage of development. Plants grown from seeds in the greenhouse and enclosed in cages often assume a spindling habit, and the symptoms of curly top on such plants are often different from those shown by plants naturally infected in the field.

**MORACEAE, MULBERRY FAMILY**

On Japanese hop (*Humulus japonicus*) infected with curly top no symptoms were apparent.

**CHENOPodiaceae, GOOSEFOOT OR SALTbUSH FAMILY**

Common summer cypress (*Kochia trichophylla*) infected with curly top showed severe foliage symptoms and developed secondary shoots from the axils of the leaves. The terminal leaves of the branches and axillary shoots were dwarfed and twisted, sometimes in a spiral. The margin and midrib of the leaves were sinuous, with knot-like swellings on the latter. The leaves showed blister-like elevations on the lower surface and cleared veinlets.

**AMARANTHACEAE, AMARANTH FAMILY**

*Amaranthus aurora* infected with curly top was stunted, having shortened internodes and secondary shoots arising from the axils of the leaves. The terminal leaves of the main and secondary shoots were dwarfed, with the outer margin rolled inward, and with the petioles often bent downward or twisted (plate 1, A). The lateral veins were sinuous, and developed knot-like swellings, and the veinlets were transparent.

On Josephs-coat (*Amaranthus gangeticus*) the symptoms of curly top were somewhat similar to those described on *A. aurora*. The leaf tissue between the protruding lateral veins was sunken in such a manner that the leaf resembled a corkscrew (plate 1, B).

On love-lies-bleeding (*Amaranthus caudatus*) the symptoms of curly top were similar to those described on *A. aurora*. Infected plants kept out of doors developed a compact mass of dwarfed, curled leaves at the apexes of the stems.

Feather cockscomb (*Celosia argentea*) infected with curly top was stunted and developed numerous axillary shoots. The pluming heads were dwarfed and had few branches (fig. 1), which were dark red or brownish red in color instead of the normal scarlet.

On common globe-amaranth (*Gomphrena globosa*) the symptoms of curly top were similar to those described on *Amaranthus aurora*.

**NYCTAGINACEAE, FOUR-o'CLOCK FAMILY**

Common four-o'clock (*Mirabilis jalapa*) infected with curly top was stunted and developed dark, dull green leaves on the axillary shoots. The youngest leaves were cupped outward and the veinlets were transparent.

## PORTULACACEAE, PURSLANE FAMILY

Common portulaca (*Portulaca grandiflora*) infected with curly top was stunted and chlorotic, bearing numerous axillary dwarfed leaves.

Common rockpurslane (*Calandrinia grandiflora*) infected with curly top was stunted, the older leaves cupped outward, and the younger dwarfed leaves formed a compact mass of curled leaves (fig. 2).



Fig. 1.—Feather cockscomb (*Celosia argentea*): left, conical pluming head from healthy plant; right, dwarfed pluming heads from two plants infected with curly top, showing reduction in number and size of branches.

## CARYOPHYLLACEAE, PINK FAMILY

Sweet william (*Dianthus barbatus*) infected with curly top was stunted, and had shortened internodes. The younger leaves at the apexes of the shoots were dwarfed, yellow, and curled, while the somewhat older leaves were often twisted (fig. 3) and had sinuous veins, cleared veinlets, and protuberances on the lower surface.

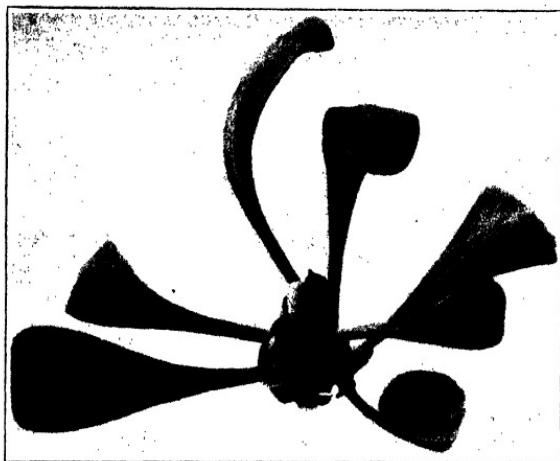


Fig. 2.—Common rockpurslane (*Calandrinia grandiflora*) infected with curly top, showing stunted plant with the older leaves cupped outward and the younger leaves forming a compact mass of curled leaves.



Fig. 3.—Sweet william (*Dianthus barbatus*): left, tip of a shoot from a healthy plant; right, apical end of a shoot from a plant infected with curly top, showing dwarfed, curled, younger leaves, and twisted older leaves.

Chinese pink (*Dianthus chinensis*) infected with curly top was stunted and developed secondary shoots from the axils of the leaves (fig. 4, A). The older leaves were curled downward (fig. 5, A), sometimes in a circle or spiral (fig. 4, A); they became chlorotic in the later stages of the



Fig. 4.—A, Chinese pink (*Dianthus chinensis*): left, plant experimentally infected with curly top by infective beet leafhoppers, showing axillary shoots with dwarfed and twisted leaves. The older leaves are curled downward, sometimes in a circle or spiral. Right, healthy check or control plant on which non-infective leafhoppers had fed. B, Spiderflower (*Cleome spinosa*) infected with curly top, showing secondary shoots forming a cluster near the apical end of the plant owing to shortened internodes. The leaves at the crown of the plant are dwarfed and cupped inward. The petioles of the older leaves are bent down.

disease, and usually had deep green veins. The leaves on the axillary shoots were dwarfed and twisted (fig. 4, A). Protuberances were present on the lower surface of the leaves.

Heddewig pink (*Dianthus chinensis* var. *heddewigii*) infected with curly top was stunted, with shortened internodes, and numerous axillary shoots. The leaves were curled downward and were often twisted. Protuberances resembling tiny warts were present on the lower surface of the leaves with pits or depressions on the upper surface of the leaves corresponding to the position of the small elevations on the under side.

On grass pink (*Dianthus plumarius*) the symptoms of curly top were similar to those described for Chinese pink (*D. chinensis*).

Carnation (*Dianthus caryophyllus*) developed leaves at the apex of infected plants and on the secondary shoots which were chlorotic and curled downward.

Babysbreath (*Gypsophila paniculata*) infected with curly top showed no symptom of the disease under greenhouse conditions.



Fig. 5.—*A*, Chinese pink (*Dianthus chinensis*) infected with curly top, showing stunted plant with older leaves curled downward and with dwarfed, filamentous, terminal leaves. *B*, Common mignonette (*Reseda odorata*): left, floral inflorescence from a plant infected with curly top, showing flowers with sepals and no corollas; right, stem from an infected plant showing axillary shoots.

Drooping catchfly (*Silene pendula*) infected with curly top developed young leaves which were dwarfed, twisted, sometimes folded inward along the midrib, and had knot-like swellings on the sinuous veins.

Maltese cross (*Lychnis chalcedonica*) infected with curly top developed numerous secondary shoots with dwarfed terminal leaves showing an inward curl or rolling of the outer margin. The veins were distorted with knot-like swellings, and wart-like protuberances occurred on the lower surface of the leaves. The veinlets on the youngest leaves were cleared.

On Haage campion (*Lychnis haageana*) the symptoms of curly top were similar to those described on Maltese cross.

## RANUNCULACEAE, CROWFOOT FAMILY

Poppy anemone (*Anemone coronaria*) infected with curly top was stunted and chlorotic. The leaves were cupped downward (fig. 6) with petioles often twisted.

Love-in-a-mist (*Nigella damascena*) infected with curly top showed a marked stunting of the crown and developed dwarfed, yellow leaves, while the petioles of the older leaves were often curled downward (fig. 7).



Fig. 6.—Poppy anemone (*Anemone coronaria*): upper row, three leaves from a plant experimentally infected with curly top, showing downward-cupped leaves; lower row, two leaves from a healthy plant.

Older infected plants developed axillary shoots, which again developed secondary shoots forming a dense cluster. In the later stage of the disease, the plants were chlorotic.

Orange larkspur (*Delphinium nudicaule*) infected with curly top failed to develop symptoms of the disease under greenhouse conditions.

## PAPAVERACEAE, POPPY FAMILY

Iceland poppy (*Papaver nudicaule*) infected with curly top had the youngest leaves dwarfed and either cupped inward or outward (plate 2, B). A yellow discoloration developed between the veins of the older leaves, while the area in the vicinity of the veins retained the green color for a long time (plate 2, B).

The varieties of Oriental poppy (*Papaver orientale*) infected with curly top included an unnamed variety, the horticultural variety



Fig. 7.—Love-in-a-mist (*Nigella damascena*) : upper, three leaves from a plant infected with curly top, showing crooked petioles; lower, terminal shoot, showing marked stunting of the crown and dwarfed leaves.

Salmon Queen, and an Oriental poppy hybrid. All were stunted and chlorotic and had leaves cupped inward along the midrib.

#### CAPPARIDACEAE, CAPER FAMILY

Spiderflower (*Cleome spinosa*) infected with curly top was stunted, with shortened internodes, and developed secondary shoots from the axils of the leaves which formed a cluster near the apical end of the



Fig. 8.—Spiderflower (*Cleome spinosa*): upper, two leaves from a healthy plant; lower, six leaves from a plant infected with curly top showing discoloration, from mottling to yellowing.

plant (fig. 4, B). The petioles of the older leaves often drooped (fig. 4, B). The leaves on the axillary shoots and on the apical end of the plant were dwarfed, curled inward (fig. 4, B and plate 1, C), and the tissue between the protruding lateral veins was sunken. The veinlets were transparent. In the later stages of the disease the leaves became mottled and chlorotic (fig. 8).

#### CRUCIFERAE, MUSTARD FAMILY

On annual stock (*Matthiola incana* var. *annua*) the most conspicuous symptom of curly top was the light-brown exudations from the petioles and leaves, which later became dark brown in color. The plants were stunted and had shortened internodes and numerous axillary shoots bearing dwarfed leaves at the apical end of the plant. The lower leaves were dry and the upper older leaves thick and leathery. Young plants infected with curly top failed to develop flowers as they matured.

Dames rocket (*Hesperis matronalis*), infected with curly top, was stunted and had numerous secondary shoots arising from the axils of the leaves. The younger leaves and those of the axillary shoots were dwarfed and formed a compact mass, from which projected the older, apparently normal leaves. The veinlets of the younger leaves were transparent.

Honesty (*Lunaria annua*) infected with curly top showed no symptoms of the disease under greenhouse conditions.

Purple candytuft (*Iberis umbellata*) infected with curly top showed no symptoms of the disease under greenhouse conditions.

#### RESEDACEAE, MIGNONETTE FAMILY

Common mignonette (*Reseda odorata*) infected with curly top developed numerous secondary shoots near the growing tips of the stems (fig. 5, B, p. 275), bearing dwarfed leaves curled downward, with sinuous margins and distorted veins. Protuberances were present on the lower surface of the older leaves. The inflorescence showed flowers with sepals, but no corollas (fig. 5, B) or with withered corollas.

#### GERANIACEAE, GERANIUM FAMILY

Fish geranium (*Pelargonium hortorum*), grown from cuttings and infected with curly top, was stunted and developed an inward rolling of the basal margin and an inward or outward cupping of the leaves. The youngest leaves showed cleared veinlets and protuberances on the lower surface. In the later stages of the disease, the lower leaves became chlorotic. Single giant-flowering hybrid geraniums grown from seeds infected with curly top developed symptoms similar to those on fish geranium.

#### TROPÆOLACEAE, TROPÆOLUM FAMILY

Canary nasturtium (*Tropaeolum peregrinum*) infected with curly top, showed axillary shoots, twisted petioles, and dwarfed curled younger leaves (fig. 9). Near the terminal ends of the branches, the lobes of the older leaves were often curled.

#### EUPHORBIACEAE, SPURGE FAMILY

Snow-on-the-mountain (*Euphorbia marginata*) infected with curly top showed cleared veinlets on the younger, dwarfed, inward-cupped leaves (fig. 10). The older leaves were cupped outward and drooped from the stem, and in the later stages of the disease turned yellow.



Fig. 9.—Canary nasturtium (*Tropaeolum peregrinum*): left, apical shoot from a plant infected with curly top, showing small secondary shoots arising from the axils of the leaves; dwarfed, curled younger leaves; curled lobes of older leaves; and twisted petioles. Right, healthy check or control plant on which noninfective beet leafhoppers had fed.



Fig. 10.—Snow-on-the-mountain (*Euphorbia marginata*): left, apical end of a branch from a healthy plant; right, apical end of a branch from a plant infected with curly top, showing cleared veinlets on the younger dwarfed, inward-cupped leaves. The older leaves are cupped outward and the lower leaves droop from the stem.

## SAPINDACEAE, SOAPBERRY FAMILY

Balloonvine (*Cardiospermum halicacabum*) infected with curly top was stunted and developed secondary shoots from the axils of the leaves. Infected plants showed severe foliage symptoms. The leaves of the terminal,



Fig. 11.—Balloonvine (*Cardiospermum halicacabum*): branch from a plant infected with curly top, showing severe foliage symptoms. The leaves of the terminal, lateral, and axillary shoots are dwarfed, curled, and twisted in clumps. The margins of the older leaves are rolled toward the midrib, with the tissue sunken between the lateral veins resulting in protruding veins.

lateral, and axillary shoots were dwarfed, curled, and twisted in clumps (fig. 11). The margin of the older leaves was rolled toward the midrib, and the tissue was sunken between the lateral veins, which resulted in protruding distorted veins (fig. 11). Wart-like protuberances were present on the lower surface of the leaves.

## MALVACEAE, MALLOW FAMILY

Herb treemallow (*Lavatera trimestris*) infected with curly top was stunted, and developed outward-cupped leaves showing transparent venation. The plants were very susceptible to the disease and wilted and died prematurely.

## VIOLACEAE, VIOLET FAMILY

Tufted pansy (*Viola cornuta*) infected with curly top was stunted, had shortened internodes, and developed secondary shoots from the axils of the leaves. The leaves of the axillary shoots were dwarfed and sometimes cupped along the midrib, or the tips of the leaves were rolled toward the midrib.

## ONAGRACEAE, EVENING-PRIMROSE FAMILY

Clarkia (*Clarkia elegans*) infected with curly top was stunted and had shortened internodes. The youngest leaves were dwarfed, sometimes twisted, or with the outer margin rolled inward. The older leaves often drooped.

## UMBELLIFERAE, PARSLEY FAMILY

Blue laceflower (*Trachymene caerulea*) infected with curly top was stunted and chlorotic. The leaves at the apical end of the plant were cupped outward and sometimes the cupping continued until each leaf resembled a small ball. The petioles were bent downward and became dry (plate 4, *B*) and often the entire plant wilted and died prematurely. The flowers were dwarfed and malformed (plate 4, *C*).

## PRIMULACEAE, PRIMROSE FAMILY

Top primrose (*Primula obconica*) infected with curly top was stunted. The youngest leaves were dwarfed, cupped outward, and chlorotic between the veins, while the area in the vicinity of the veins remained green. The veinlets were transparent.

*Primula saxatalis* infected with curly top was stunted, and the youngest leaves were cupped outward (fig. 12, *A*).

Cowslip primrose (*Primula veris*) infected with curly top was stunted and chlorotic.

Oxlip primrose (*Primula elatior*) infected with curly top was stunted and chlorotic, often wilted, and died prematurely.

## PLUMBAGINACEAE, PLUMBAGO OR LEADWORT FAMILY

On notchleaf sea-lavender (*Limonium sinuatum*), the youngest leaves of infected plants were dwarfed and chlorotic.



Fig. 12.—A, *Primula saxatalis*: leaves from a plant infected with curly top, showing outward-cupped leaves. B, Nest-egg gourd (*Cucurbita pepo* var. *ovifera*): axillary shoots from an infected plant, showing dwarfed, puckerered, balled leaves, with shortened petioles, and with dwarfed flowers. C, Spoon gourd (*Lagenaria leucantha*): terminal shoot from an infected plant, showing outward-cupped leaves, and dwarfed tendrils.

## APOCYNACEAE, DOGBANE FAMILY

Madagascar periwinkle (*Vinca rosea*) infected with curly top showed no symptoms under greenhouse conditions.

## CONVOLVULACEAE, MORNING-GLORY FAMILY

Crimson starglory (*Quamoclit lobata*) infected with curly top showed an inward rolling of the margin of the older leaves with wart-like pro-

tuberances on the lower surface (plate 2, C). The younger leaves were twisted and developed bent petioles.

Brazilian morning-glory (*Ipomoea setosa*) infected with curly top showed an inward rolling of the margin of the older leaves, puckering of the blades, transparent venation (plate 3, A), and sometimes wart-like protuberances on the lower surface of the leaves. The younger leaves were sometimes folded inward along the midrib (plate 3, A).

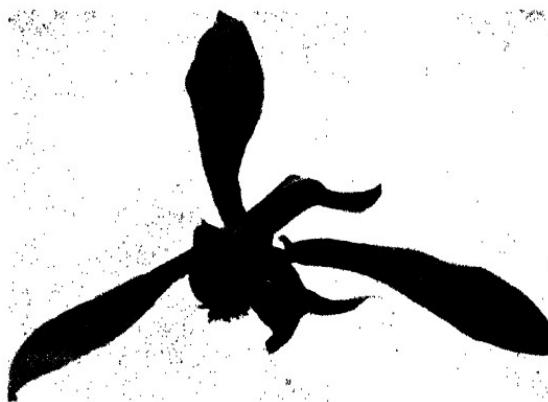


Fig. 13.—Drummond phlox (*Phlox drummondii*) infected with curly top, showing stunted plant, with shortened internodes, and dwarfed, curled leaves.

#### POLEMONIACEAE, PHLOX FAMILY

Drummond phlox (*Phlox drummondii*) infected with curly top was stunted, with shortened internodes (fig. 13), and had secondary shoots arising from the axils of the leaves. The leaves on the axillary shoots were dwarfed, linear near the tips, cupped inward, and chlorotic. The midrib of the older leaves was sinuous and the veinlets were transparent. The flowers near the apexes of the secondary shoots developed sepals but no petals.

On purplebell cobaea (*Cobaea scandens*) infected with curly top the apical leaflets were dwarfed and yellow, their protruding lateral veins resembled a corkscrew, and the petioles bent downward. The somewhat older leaves showed clear veinlets.

#### HYDROPHYLACEAE, WATER-LEAF FAMILY

Spotted nemophila (*Nemophila maculata*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

## BORAGINACEAE, BORAGE FAMILY

Alkanet (*Anchusa azurea*) infected with curly top was stunted. Its youngest leaves were dwarfed.

Chinese forget-me-not (*Cynoglossum amabile*) infected with curly top was stunted, had shortened internodes, and developed axillary shoots. The youngest leaves were dwarfed, curled, and twisted (fig. 14).



Fig. 14.—Chinese forget-me-not (*Cynoglossum amabile*): left, terminal and lateral shoots from a healthy plant; upper right, flower stalks forming a dense cluster at the terminal end of a branch from an infected plant; lower right, lateral shoot, showing dwarfed, curled, and twisted leaves.

Brown droplets exuded from the leaves and stem. The flower stalks from numerous axillary shoots formed a dense cluster (fig. 14) and the flowers were frequently dwarfed and reduced in number.

True forget-me-not (*Myosotis scorpioides*) infected with curly top was stunted, and developed numerous secondary shoots. The youngest leaves were dwarfed, almost linear in shape near the apexes of the shoots, and chlorotic. The older leaves were curled downward with the

margin cupped inward. The leaves on the secondary shoots were dwarfed and had a sinuous midrib and protuberances on the veins.

Common heliotrope (*Heliotropium peruvianum*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

#### LABIATAE, MINT FAMILY

Scarlet sage (*Salvia splendens*) infected with curly top and kept out of doors was stunted, had shortened internodes, and developed a compact mass of axillary shoots. The margins of the leaves were often rolled in-



Fig. 15.—Scalloped salpiglossis (*Salpiglossis sinuata*) infected with curly top, showing secondary shoots arising from the axils of the leaves and base of the plant. The leaves of the apical end of the plant and on the axillary shoots are rolled or curled inward. The older leaves are cupped outward.

ward (plate 3, C), or cupped inward along the midrib, the veinlets were transparent, and the petioles were bent downward. The leaves near the tips of the secondary shoots were dwarfed and chlorotic. The flowers were reduced in number, dwarfed, often failed to expand, and some remained green instead of the normal brilliant scarlet color.

#### SOLANACEAE, NIGHTSHADE FAMILY

Jasmine tobacco (*Nicotiana alata* var. *grandiflora*) infected with curly top was stunted and had shortened internodes. The youngest leaves were dwarfed, cupped outward, and had cleared veinlets.

Common petunia (*Petunia hybrida*) (Rosy Morn variety) infected with curly top was stunted and developed numerous secondary shoots bearing dwarfed leaves. Protuberances were present on the veins on the lower surface of the leaves of the secondary and apical shoots and gave the veins a roughened appearance. The corolla of the flowers often failed to expand and became dry. In the later stages of the disease the entire plant turned yellow and died.

Scalloped salpiglossis (*Salpiglossis sinuata*) infected with curly top was stunted and had secondary shoots arising from the axils of the leaves



Fig. 16.—Wiseton butterflyflower (*Schizanthus wisetonensis*): shoots and leaves from a plant infected with curly top, showing semicircular and circular curling of the petioles of the younger leaves.

and base of the plant (fig. 15). The dwarfed, chlorotic leaves on the terminal end of the plant and on the axillary shoots were rolled or curled inward. The older leaves were cupped outward. The veinlets were transparent (plate 3, B) and wart-like protuberances were present on the lower surface of the leaves.

On *Browallia speciosa* the terminal leaflets of infected plants were dwarfed, cupped inward along the midrib, and had protruding lateral veins. The veinlets were transparent at the base of the older leaves, and the petioles were bent downward.

Wiseton butterflyflower, *Schizanthus wisetonensis* (*S. pinnatus* × *S. grahamii*) infected with curly top showed a circular or semicircular curling of the petioles of the younger leaves (fig. 16). The leaves on the secondary shoots arising from the axils of the leaves were dwarfed and had curled petioles. Infected plants often wilted and died prematurely.

## SCROPHULARIACEAE, FIGWORT FAMILY

Yellow foxglove (*Digitalis ambigua*) infected with curly top developed youngest leaves which were dwarfed, cupped inward along the midrib, and the veinlets were transparent.

Kenilworth ivy (*Cymbalaria muralis*) infected with curly top developed a large number of dwarfed leaves on the secondary shoots at the

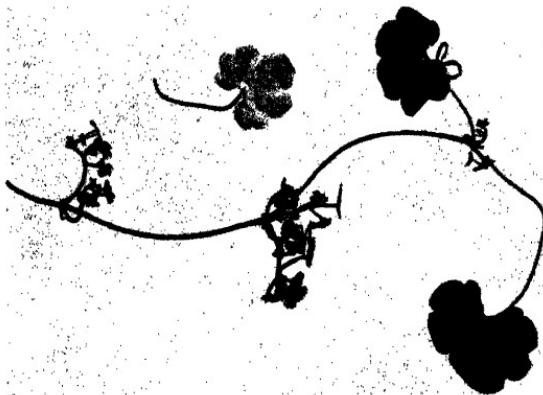


Fig. 17.—Kenilworth ivy (*Cymbalaria muralis*): apical end of branch from a plant infected with curly top, showing dwarfed leaves on the secondary shoots at the nodes and bent and looped petioles.

nodes (fig. 17) instead of rooting at the nodes. The larger leaves at the apexes of the branches were slightly cupped inward with wart-like protuberances on the lower surface of the leaves and with bent or looped petioles (fig. 17).

Pouched nemesia (*Nemesia strumosa*) infected with curly top was stunted and chlorotic. The veinlets were transparent on the youngest leaves.

Golden monkeyflower (*Mimulus luteus*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

## ACANTHACEAE, ACANTHUS FAMILY

Black-eyed clockvine (*Thunbergia alata*) infected with curly top developed secondary shoots with dwarfed, puckered, yellow leaves (plate 2, A). The veinlets were transparent (plate 4, E). The petioles were often bent downward.

## CUCURBITACEAE, GOULD FAMILY

The ten ornamental gourds listed in table 2 were experimentally infected with curly top. The symptoms of curly top were the same on all ten. The plants were stunted and developed secondary shoots bearing chlorotic, dwarfed, puckered, outward-cupped leaves with shortened petioles (fig. 12, B, p. 283). The leaves on the secondary shoots resembled a small ball.



Fig. 18.—Cardinalflower (*Lobelia cardinalis*): left, healthy plant; right, plant infected with curly top, showing dwarfed, inward-curled young leaves and malformed, twisted, older leaves.

The older leaves were cupped outward (fig. 12, C), and dull green. The tendrils usually were dwarfed. The veinlets were transparent. The flowers in the axils of the leaves were sessile, and their corollas failed to expand and often withered prematurely.

## LOBELIACEAE, LOBELIA FAMILY

White-eye lobelia (*Lobelia erinus* var. *speciosa*) infected with curly top was stunted, and had numerous secondary shoots bearing dwarfed, chlorotic leaves.

Cardinalflower (*Lobelia cardinalis*) infected with curly top was stunted. The youngest leaves were dwarfed, puckered, with inward-curled margins and cleared veinlets. The oldest leaves were malformed and twisted (fig. 18).

## COMPOSITAE, COMPOSITE FAMILY

Crowndaisy (*Chrysanthemum coronarium*) infected with curly top was stunted, chlorotic, and had dwarfed, curled leaves.

Feverfew (*Chrysanthemum parthenium*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

Marguerite (*Chrysanthemum frutescens*) infected with curly top developed a dense cluster of dwarfed, chlorotic leaves at the apexes of the stems and secondary shoots. The leaflets were curled inward, outward, or folded inward along the midrib with bent petioles (plate 4, D).

Scentless false-camomile (*Matricaria inodora*) infected with curly top was stunted, chlorotic, and developed numerous secondary shoots arising from the axils of the leaves. The youngest leaves were dwarfed, curled, and twisted.

Cucumber sunflower (*Helianthus debilis*) infected with curly top developed chlorotic, secondary shoots from the axils of the leaves. The flowers were dwarfed (plate 4, A).

Golden thinleaf sunflower (*Helianthus decapetalus* var. *multiflorus*), infected with curly top failed to show symptoms under greenhouse conditions.

All varieties of common zinnia (*Zinnia elegans*) grown in California were experimentally infected with curly top. Infected plants were stunted, and had inward-cupped leaves showing cleared veinlets.

On orange zinnia (*Zinnia haageana*) the symptoms were similar to those described on common zinnia.

The cosmos varieties and species infected with curly top were common cosmos (*Cosmos bipinnatus*), giant-flowering cosmos (*Cosmos bipinnatus*) and crested cosmos (*Cosmos hybridus*). The symptoms on the three were similar. Infected plants were stunted, chlorotic, and had shortened internodes and secondary shoots arising from the axils of the leaves. The leaflets were curled and twisted with the petioles sometimes bent downward.

Calliopsis (*Coreopsis tinctoria*) infected with curly top developed secondary shoots with circular twisted leaves. The leaves on other portions of the plants showed no symptoms of the disease except drooping of the petioles. The terminal flower heads on the secondary shoots were dwarfed.

On English daisy (*Bellis perennis*) the youngest leaves infected with curly top were dwarfed, malformed, and cupped inward. The veinlets were transparent, and wart-like protuberances were present on the lower surface of the leaves.

Swan-river-daisy (*Brachycome iberidifolia*) infected with curly top developed secondary shoots from the axils of the leaves terminating in tufts of dwarfed, twisted, yellow leaves (fig. 19). A tangled mass of leaves was present at the apical end of the plant and at the end of the stunted branches. The petioles of the older leaves were often curled downward, upward, or twisted.

Bushy arctotis (*Arctotis stoechadifolia*) infected with curly top de-



Fig. 19.—Swan-river-daisy (*Brachycome iberidifolia*): branch from a plant experimentally infected with curly top, showing secondary shoots arising from the axils of the leaves and terminating in a tuft of dwarfed, twisted leaves.

veloped leaves on the secondary shoots which were dwarfed and had the lobes curled inward.

French marigold (*Tagetes patula*) infected with curly top developed axillary shoots bearing dwarfed leaves with the petioles curled downward. The flower buds were yellow instead of green and the peduncles were often curled downward.

Aztec marigold, or African marigold (*Tagetes erecta*) infected with curly top failed to show symptoms under greenhouse conditions.

Winter cape-marigold (*Dimorphotheca aurantiaca*) infected with curly top was stunted, and developed shortened internodes and axillary shoots. In the advanced stage of the disease the terminal leaves were dwarfed, curled, and yellow (fig. 20). The teeth-like projections of the leaves were often curled inward. The longitudinal veins on the lower surface of the leaves were distorted.

Pot-marigold (*Calendula officinalis*) infected with curly top failed to develop symptoms under greenhouse conditions.

Rose everlasting (*Helipterum roseum*) infected with curly top developed terminal shoots which were either erect or drooping with a cluster



Fig. 20.—Winter cape-marigold (*Dimorphotheca aurantiaca*): branch from a plant infected with curly top, showing dwarfed, curled leaves at the tip of the stem and also on the secondary shoots arising from the axils of the leaves. The teeth-like projections of the leaves are often curled inward.

of twisted yellow leaves around the bud (fig. 21). The margin of the leaves below the bud were sinuous.

When small plants of strawflower (*Helichrysum bracteatum*) were infected with curly top they were stunted, their youngest leaves were dwarfed, twisted, and yellow in the advanced stage of the disease. The somewhat older leaves were also twisted and the veinlets were transparent. Plants infected at a later stage of development had numerous secondary shoots toward the tip of the branches with twisted leaves showing knot-like swellings on the veins.

Sweet-sultan (*Centaurea moschata*) infected with curly top failed to develop symptoms of curly top under greenhouse conditions.

Basketflower (*Centaurea americana*) infected with curly top failed to show symptoms of the disease under greenhouse conditions.

On cornflower (*Centaurea cyanus*) infected with curly top, the leaves of the secondary shoots were dwarfed and sometimes two adjacent leaves were coiled downward.



Fig. 21.—Rose everlasting (*Helipterum roseum*): tips from plant infected with curly top, showing erect or drooping terminal shoots with clusters of twisted leaves around the buds.

#### LONGEVITY AND LIFE CYCLE OF THE BEET LEAFHOPPER

A comparative record was obtained of the longevity of male and female beet leafhoppers on plants on which the insects were not able to complete their life history. Adults of the spring or summer generations were used and not the dark adults of the overwintering generation. Two plants of each variety or species were inoculated with curly top by either male or female leafhoppers, and, by daily examination of each cage, a record was obtained of the longevity of the last living male and female as indicated in table 2. When the insects died after a short exposure on the host plant, presumably owing to unfavorable food, the plants were repeatedly inoculated by different lots of insects and the range in the longevity was determined. The longevity of the adults was often shorter on young plants than on older ones.

A record was kept of all host plants on which the nymphs, after hatching from the egg, completed their nymphal stages and acquired the winged stage. The host plants of curly top on which the beet leafhopper completes its life cycle are indicated in table 2. There was considerable variation in the percentage of specimens reared to the adult stage on the different host plants. This difference in population was probably due to the variation in the amount of suitable food provided by the plant.

On unfavorable food plants, the nymphs hatched but soon died. The nymphal stages were often prolonged, presumably owing to unfavorable food.

The plants which were proved susceptible to curly top were not always favorable food plants of the leafhopper. Plants which were suitable food plants and on which the insects were able to live for long periods were often found resistant or immune to curly top. On the other hand, the leafhoppers often transmitted curly top to certain plants that were unfavorable food plants, and on which they could live for only a few days.

#### SUMMARY

Curly top was experimentally transmitted to 92 species of ornamental flowering plants in 73 genera belonging to 33 families. The virus was recovered from each species or variety of infected plant by previously noninfective beet leafhoppers and transferred to sugar beets. The host plants of curly top among ornamental flowering plants include 47 species of annuals, 2 species of annuals or biennials, 11 species of perennials grown as annuals, 3 species of biennials or perennials, and 30 species of perennials.

Ornamental flowering plants infected with curly top show a variation in symptoms. Fifteen species, including 6 species of annuals, 2 species of annuals grown as perennials, and 7 species of perennials, failed to develop symptoms of the disease under greenhouse conditions.

Many of the plants infected with curly top were stunted and developed numerous secondary shoots from the axils of the leaves. Chlorosis and a development of shortened internodes was often characteristic of infected plants.

The leaves of some infected plants were dwarfed and often developed an inward rolling of the outer margin, and later the entire blade showed a pronounced inward curling toward the midrib. Other species showed an outward rolling of the leaves toward the midrib. In still other species the leaves may be cupped inward or outward, or they may be twisted, sometimes in a spiral. Puckering and mottling of the leaves occur in certain species and malformation of the leaves in others.

Many infected ornamental flowering plants developed cleared veins, a reliable symptom of curly top on the sugar beet. Another reliable symptom of curly top is the roughened appearance on the lower surface of the leaves developing after the veinlets have cleared. The veins develop numerous small elevations resembling tiny warts. As the disease progresses, nipple-like papillae and knot-like swelling resembling galls develop here and there on the distorted, thickened veins.

Some infected plants, such as annual stock (*Matthiola incana* var. *annua*) and Chinese forget-me-not (*Cynoglossum amabile*) exude droplets of brown liquid from the veins, midrib, petioles, and stems.

Wilting and premature death of the plants occur with blue laceflower (*Trachymene caerulea*), Wiseton butterflyflower (*Schizanthus wisetonensis*), and herb treemallow (*Lavatera trimestris*).

Young plants infected with curly top frequently produce no flowers as they mature. Older plants infected before blooming often develop dwarfed, malformed, and fewer flowers than healthy plants.

The life cycle of the beet leafhopper was completed on 65 species of ornamental flowering plants in 51 genera, belonging to 23 families.

#### ACKNOWLEDGMENTS

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Plate 1.—A, Terminal shoot of *Amaranthus aurora* infected with curly top, showing dwarfed, terminal leaves with the outer margin rolled inward and with the petioles bent downward or twisted. B, Terminal shoot of Joseph's-coat (*Amaranthus gangeticus*) with the tissue sunken between the lateral veins and with protuding veins resembling a corkscrew. C, Terminal shoot of spiderflower (*Cleome spinosa*) infected with curly top, showing dwarfed, inward-curled leaflets.

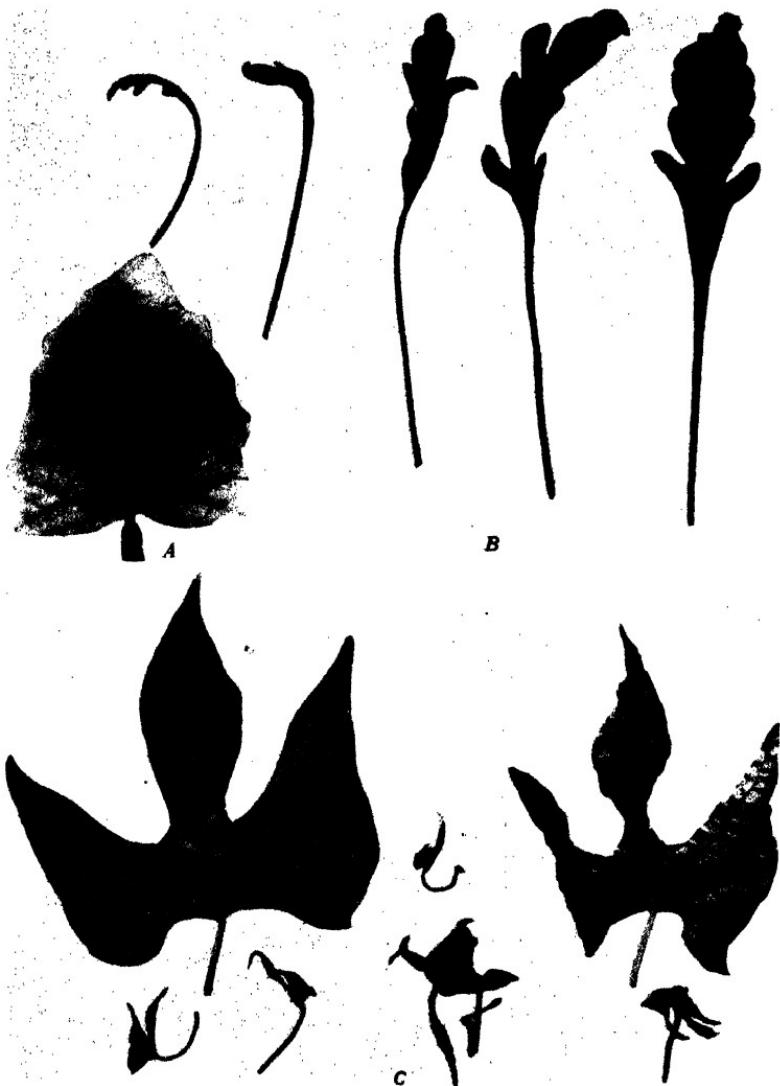


Plate 2.—*A*, Black-eyed clockvine (*Thunbergia alata*): chlorotic leaf from a plant infected with curly top. *B*, Iceland poppy (*Papaver nudicaule*): left, two small leaves from an infected plant showing outward and inward cupping; right, three leaves showing successive stages of yellowing between the veins, while the area along the veins remained green for a long time. *C*, Crimson starglory (*Quamoclit lobata*): left, large leaf from a healthy plant used as a check or control on which noninfective beet leafhoppers had fed; right, large leaf from a plant infected with curly top showing protuberances on the lower surface; small leaves showing malformations.



Plate 3.—*A*, Brazilian morning-glory (*Ipomoea setosa*): right and left leaves, from a plant infected with curly top showing inward rolling of the margin and transparent venation; lower center, leaf folded inward along the midrib; upper center, leaf from a healthy plant used as a check or control on which noninfective beet leafhoppers had fed. *B*, Scalloped salpiglossis (*Salpiglossis sinuata*): leaf showing cleared veinlets. *C*, Scarlet sage (*Salvia splendens*): apical shoot from a plant infected with curly top showing inward roll of the margin of the youngest leaves, cleared veinlets, and bent petioles.

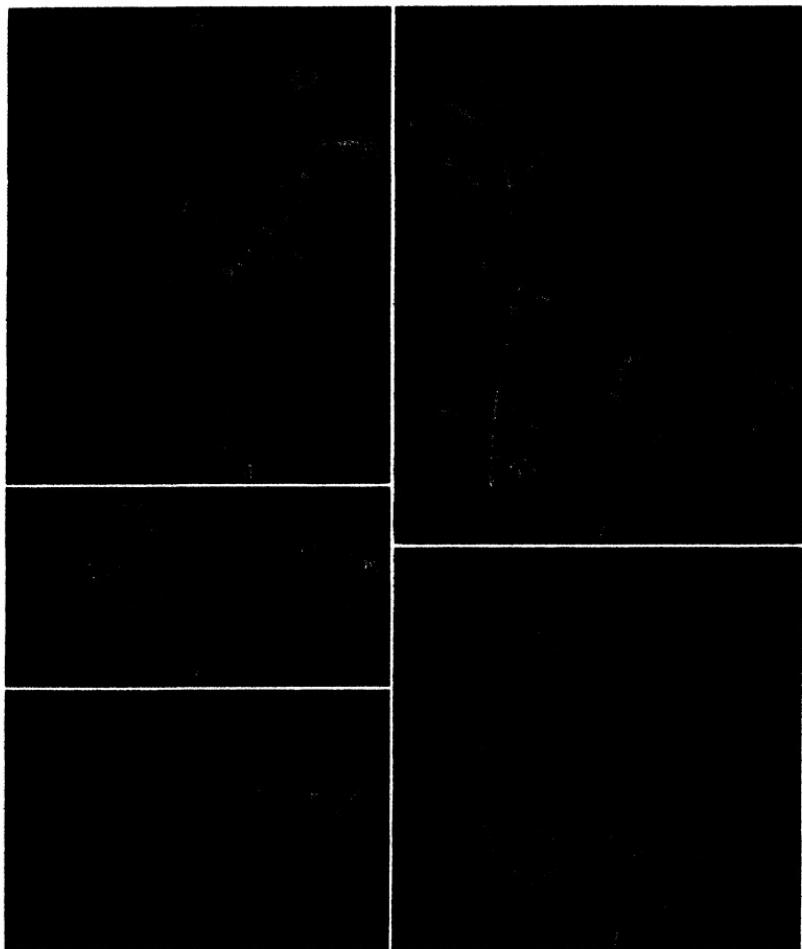


Plate 4.—*A*, Cucumber sunflower (*Helianthus debilis*): upper, flower from a healthy plant; lower, dwarfed flower from a plant infected with curly top. *B*, Blue laceflower (*Trachymene caerulea*): left, healthy plant which had been exposed to noninfective beet leafhoppers; right, stunted plant infected with curly top by infective beet leafhoppers showing outward-cupped leaves and dried petioles. *C*, Blue laceflower (*Trachymene caerulea*): left, normal flower from a healthy plant; right, dwarfed and malformed flower from an infected plant. *D*, Marguerite (*Chrysanthemum frutescens*): secondary shoots and leaves from a plant infected with curly top, showing curled leaflets. *E*, Black-eyed clockvine (*Thunbergia alata*): leaf from a plant infected with curly top showing cleared veinlets.

**NEGATIVE EVIDENCE ON MULTIPLICATION  
OF CURLY-TOP VIRUS IN THE BEET  
LEAFHOPPER, *EUTETTIX*  
*TENELLUS***

**JULIUS H. FREITAG**



# NEGATIVE EVIDENCE ON MULTIPLICATION OF CURLY-TOP VIRUS IN THE BEET LEAFHOPPER, *EUTETTIX*

TENELLUS<sup>1, 2, 3</sup>

JULIUS H. FREITAG<sup>4</sup>

## INTRODUCTION

MULTIPLICATION of certain viruses in their insect vectors and the consequent theory of biological relation between insects and viruses have been generally surmised on the basis of insufficient evidence. For many virus diseases a definite interval of time has been reported as necessary after an insect has fed on a diseased host before it is able to transmit a virus to a healthy host. This interval has been called the "incubation period" of the virus in the insect. Some writers have suggested that this may be a period during which the virus undergoes some developmental phase in a possible life cycle in the body of the insect. Insect vectors of virus diseases have also frequently been reported to remain infective during their entire adult life, after once having acquired the virus by feeding on a diseased host.

*Multiplication of Viruses in Insects.*—Few experiments have been conducted which give any definite evidence on the question of the multiplication of viruses in their insect vectors. Davis, Frobisher, and Lloyd,<sup>5</sup> working with yellow fever and the mosquito, *Aedes aegypti* L., have demonstrated that the quantity of virus present in the vector never increases beyond that found immediately after an infective meal. The quantity of virus was determined by finding the greatest dilution of the bodies of crushed mosquitoes that would give infection when inoculated into healthy animals. During the two weeks after a meal of infectious blood there occurred a reduction in the quantity of virus to approximately 1 per cent of that in recently fed insects. There was an increase in titratable virus at a later period, but they suggest that this rise signi-

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<sup>5</sup> Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

fies not a multiplication, but merely an increase in extracellular virus. At no later period did the quantity of virus equal that demonstrable in freshly fed insects, and the authors, therefore, conclude that no multiplication of the virus occurs in the yellow-fever mosquito.<sup>6</sup>

Fukushi<sup>10</sup> has obtained evidence which indicates that a multiplication of the rice-dwarf virus occurs in the insect vector *Nephrotettix apicalis* var. *cincticeps*. He demonstrated that the virus was transmitted through the eggs of the infective leafhoppers to the progeny of the third generation. Apparently the only explanation for the retention of the rice-dwarf virus by the leafhopper for three generations without having access to a source of infection is that the virus must have multiplied in the vector. This transmission of rice-dwarf virus through the egg of the infective leafhopper to its progeny is the only record of such an occurrence in plant-virus literature.

*Mechanical Transmission of Plant Viruses by Insects.*—There are several examples of the transmission of the plant viruses by insects in such brief periods as to preclude multiplication of the virus in the vector and to indicate that in these cases at least the transmission must have been purely mechanical. Doolittle and Walker<sup>11</sup> have shown that the striped cucumber beetle, *Diabrotica vittata* (Fabr.), retains the virus of cucumber mosaic for a short time only. They found that the virus-free melon aphid, *Aphis gossypii* Glover, could become infective after only 5 minutes' feeding on a cucumber-mosaic plant and could produce infection after feeding on a healthy plant for a like period. Aphids from mosaic plants do not produce the disease after 6 to 8 hours' confinement in a glass tube; this period being approximately the same as that during which expressed juice remains infective.

Although normally an "incubation period" of 1 day or longer is required before the beet leafhopper, *Eutettix tenellus* (Baker), is able to cause curly-top infection, Severin<sup>12, 13</sup> has demonstrated that when a large number of noninfective insects is used, they may on rare occasions transmit curly top after feeding for a period of 10 minutes on a diseased beet and 10 minutes on a healthy plant during high temperatures in the greenhouse. Transmissions were also obtained under similar conditions within periods of  $\frac{1}{2}$ , 1,  $1\frac{1}{2}$ , 2, 3, 4, 5, and 6 hours if the insects were fed for one-half of these periods on a diseased plant and the other half on a

<sup>6</sup> Since this paper was written, a paper by Merrill and Ten Broeck has come to my attention. They report a multiplication of the virus of equine encephalomyelitis in the mosquito vector, *Aedes aegypti*. The virus was carried from mosquito to mosquito by serial passage through seventeen lots of insects and was demonstrated to be present in undiminished amounts in the final lot of mosquitoes tested. (Merrill, M. H., and Carl Ten Broeck. The transmission of equine encephalomyelitis virus by *Aedes aegypti*. Jour. Exp. Med. 62: 687-95. 1935.)

healthy plant. The extract made from the mouth parts of leafhoppers which had fed  $\frac{1}{2}$  to 1 hour on a diseased beet was found on rare occasions to be infective. He states, "These experiments indicate that contamination of mouth parts without multiplication of the curly-top virus in the body of the insect may account for the transmission of the disease."

Swezy<sup>(27)</sup> proposed two theories to account for the transmission of curly top during short periods by the beet leafhopper: (1) an abnormal condition of the alimentary canal consisting of a clump of bacteria in the lumen of the esophagus anterior to the esophageal valve might hinder the free passage of food, and infected beet juice might, therefore, be regurgitated through the mouth parts a short time after being taken in; (2) the passage of the infective organism unchanged through the body of the insect to the salivary glands and its expulsion in the saliva to cause infection. A third theory proposed "that a change in life cycle of the infective organism occurs in the body of the insect and this must be completed before the insect is capable of readily transmitting the disease to a healthy plant."

*Period of Delay in Development of Infective Capacity in the Insect.*—Severin<sup>(16)</sup> defined the virus "incubation period" in the beet leafhopper as "the time for the infective principle to pass into the mouth parts, alimentary canal, blood, salivary glands and out of the mouth parts in sufficient quantity to produce infection." He found that a low percentage of single leafhoppers were able to acquire and transmit the virus under the high temperatures in the greenhouse, during as short a period as 7 hours. Infections were also produced by single insects after periods varying from 9 to 23 hours.

Smith and Boncquet<sup>(22)</sup> reported that approximately 3 hours of feeding were necessary before the insect could obtain the infective agent and that an "incubation period" of from 24 to 48 hours was necessary before the beet leafhopper could transmit curly top of sugar beets. These facts suggested to them that curly-top virus was not merely transferred by a mechanical process, but that some development or change took place within the body of the insect during the first few hours after feeding on a diseased plant.

Carsner and Stahl<sup>(16)</sup> found that single beet leafhoppers were able to transmit curly top within a period of  $21\frac{3}{4}$  hours after first feeding on a diseased beet. They reported that "a greater number of insects become able to transmit the virus after a longer period than 24 hours than are able to do so in the shorter time. The facts cited seem to indicate that a multiplication of the causal agent takes place within the insects."

Kunkel,<sup>(28)</sup> working in New York, demonstrated that the "incubation

period" of the aster-yellows virus in *Cicadula divisa* Uhl. [*C. sexnotata* (Fall.)] varied from 10 to 19 days. He also showed<sup>(19)</sup> that this period varied from 17 to 26 days with the California aster-yellows virus in the same species of leafhopper. He states, "It is difficult to conceive that any agent other than a living organism would require an incubation period in the insect carrier."

Bald and Samuel<sup>(19)</sup> showed that in the transmission of spotted wilt of tomato there was a delay of 5 to 7 days in the development of the infective capacity of the black carnation thrips, *Frankliniella insularis* (Franklin).

In his work with yellow spot of pineapple, Linford<sup>(19)</sup> reported that a period of approximately 10 days must elapse after onion thrips, *Thrips tabaci* Lindeman, have fed on a diseased plant before they become infective.

Elze<sup>(19)</sup> demonstrated that the period between the first feeding on a diseased leafroll potato plant and the development of infectivity in the peach aphid, *Myzus persicae* (Sulzer), ranged from 24 to 38 hours.

Smith<sup>(20)</sup> considered the minimum "incubation period" of the virus of leafroll of potato in the peach aphid to be 54 hours.

Storey<sup>(21)</sup> found that the "incubation period" of the virus of streak of maize in the leafhopper, *Cicadulina mbila* (Naude), was from 6 to 63 hours at 30° C. In some experiments 84 hours elapsed before the previously noninfective insects produced an infection after having first fed on a diseased plant. He states, "There was a great irregularity in the behavior of individual leafhoppers which makes impossible any exact numerical expression of the duration of the uninfective period."

*Retention of Plant Virus by Insects.*—The fact that insect vectors of viruses often retain their infective power over long periods of time has been considered as evidence in favor of the theory that a virus multiplies in the body of the insect. According to the literature, there are, however, some insect vectors of viruses which lose the infective capacity when they do not have access to a source of virus.

Bonequet and Stahl<sup>(22)</sup> asserted that the ability of the beet leafhopper to transmit curly top was lost in 15 to 35 days if the insects were transferred daily to a healthy beet.

Carsner<sup>(23)</sup> stated that infective beet leafhoppers after being kept on cattle spinach, *Atriplex polycarpa*, which is nonsusceptible to curly top, retained their infectivity for a period of 58 days.

Sererin<sup>(24)</sup> found that three infective male beet leafhoppers infected in the first nymphal instar retained their infectivity during all of the nymphal stages and during their entire adult life of 101, 103, and 105

days, respectively. Eight infective males each provided with a healthy beet daily during its adult life transmitted the virus to from 16.2 to 55.5 per cent of the beets. These males usually transmitted curly top to fewer beets toward the end of their natural life.

Kunkel,<sup>41</sup> working in New York with aster yellows, showed that 3 of 6 adult *Cicadula divisa* retained their infective capacity for a period of 100 days when transferred at intervals to healthy asters. The other 3 insects did not infect any plants during the last 3 weeks of the experiment. He confined infective insects on rye plants, which are immune to aster yellows, for at least 2 months and demonstrated that the leafhoppers had not lost the infective principle during this period. He found, however, that while some specimens retained the infectivity throughout life, others seemed to lose it after a short time.

In Australia, Samuel, Bald, and Pittman<sup>42</sup> demonstrated that the black carnation thrips, the vector of spotted wilt of tomato, when placed on a healthy plant each day, did not lose the power to cause infection during the 24 days of the experiment, which included the pupal period. The insects, however, did not infect every plant on which they fed.

Elze<sup>43</sup> found that the peach aphid, the vector of leafroll of potato, does not lose its power to infect during the process of molting. When infective aphids were transferred to a nonsusceptible host plant such as spinach for 7 to 10 days, they were able to infect healthy potatoes.

Smith<sup>44</sup> reported that infective peach aphids retained the virus of leafroll of potato for a period of 7 days while feeding on immune plants such as cabbage. He said, "It seems probable, but this has not been proved, that *Myzus persicae* once infected remains so for the rest of its natural life."

In the case of curl of raspberry, Bennett<sup>45</sup> stated that the aphid vector, *Aphis rubiphila* Patch, does not lose its power to infect when kept on plants immune to curl for a period of 3 weeks and probably for the life time of the insect.

Bennett<sup>46</sup> reports that the aphid vector of red-raspberry mosaic, *Amphorophora rubi* (Kaltenbach), acquired the virus in 12 hours or less and transmitted it to healthy plants during the following 12-hour period, but when this aphid was transferred to successive healthy plants, it lost the mosaic virus.

Storey<sup>47</sup> showed that three single specimens of *Cicadulina mbila* retained the power to transmit the virus of streak of maize for periods of at least 84, 111, and 116 days, when provided with a healthy maize plant daily. One of eight insects tested, however, after infecting 16 of the first 33 plants, showed a progressive weakening of the infective power, and it

infected plants, thereafter, only on the 42nd, 45th, and 66th day and failed to produce an infection during the last 45 days of its life. The author states that "some specific biological relationship exists between *Cicadulina mbila* and streak virus. The evidence clearly indicates a multiplication of the virus in the insect."

Storey<sup>(20)</sup> later reported that insects infected by feeding upon streak-diseased maize normally continued to cause infections up to the time of their death. However, insects infected by inoculating them with streak virus by means of a needle puncture in their abdomen, ultimately became noninfective if kept on healthy plants. Plants infected by insects inoculated with the virus by the needle-puncture method developed symptoms of the disease more slowly than those infected by insects which had fed on diseased plants. Insects which had lost the power to cause infection were reinfected either by feeding them on a diseased plant or by needle inoculation, and they were then capable of again causing infection. Storey stated, "On the whole, the available evidence rather suggests that the loss is due to an exhaustion of the supply of virus in the insect."

An investigation was undertaken to determine whether the curly-top virus multiplies in the beet leafhopper. Since we have been unable to carry out experiments which would give direct evidence regarding multiplication of the virus in the leafhopper, an attempt was made to perform experiments which could give indirect evidence on this question. Such evidence might be obtained if it could be shown whether the insects retained the infective capacity during their entire adult life, and whether leafhoppers fed for short periods on a diseased beet could cause as many infections as those fed for longer periods on a source of virus. Transmission experiments were performed to ascertain the number of infections produced, and the longevity of the virus in the leafhopper during adult life, after having fed for varying periods on curly-top beets. Experiments were also conducted on the transmission of curly top by male and female leafhoppers, the ability of the leafhopper to acquire and transmit the virus during various periods of adult life, and whether an insect could be reinfected with the virus during later adult life. A comparison was made of the incubation period of the disease in sugar beets infected by the insects fed for short periods with those fed during the nymphal stages on curly top beets.

## GENERAL METHODS

In all transmission experiments in which the beet leafhoppers were transferred daily to healthy beets, each specimen was confined in a small cylindrical cage  $7\frac{1}{2}$  inches in height by  $5\frac{1}{2}$  inches in diameter constructed of wood with top and sides covered with lawn except for a glass plate 3 by 6 inches through which observations were made (fig. 1). The bottom of the cage was made of wood and had a small circular opening 1 inch in diameter through which the beet seedling was inserted. The wooden frame and bottom of the inside of the cage was painted black so that the adults could readily be observed during the transfers. To prevent the escape of the insect from the cage enclosing the beet seedling, the soil in the pots was covered with a layer of coarse dry sand and the bases of the petioles were surrounded with cotton (fig. 1, C). The adults were transferred from one cage to another by capturing them with a glass pipette (fig. 2).

Curly-top beets used as a source of virus in the experiments reported in this paper were severely affected and were obtained from the San Joaquin Valley and interior regions of the Salinas Valley. During the four seasons 1931-1935 in which these experiments were performed, the beet leafhoppers were always infected with the virus by feeding them on curly-top beets in an advanced stage of the disease. Reliable symptoms of curly top, such as cleared veinlets and protuberances on the lower surface of the leaves, were present on all beets used as a source of virus in the various experiments.

Healthy sugar-beet seedlings with from 6 to 12 leaves were used in all experiments. The beet seedlings were grown in 4-inch clay pots and were kept out of doors in insect-proof cages.

The infective beet leafhoppers were reared during their nymphal stages on curly-top beets, the nymphal period requiring from 26 to 36 days in the greenhouse, as determined by Severin.<sup>17</sup>

Noninfective beet leafhoppers were obtained by transferring recently hatched nymphs before feeding from a diseased to a healthy sugar beet by means of a camel's-hair brush, as first described by Stahl and Carsner.<sup>18</sup> A supply of noninfective leafhoppers was reared on healthy beets in large cages in the greenhouse. The healthy beets were kept in insect-proof cages to protect them from accidental infection with curly top. Each cage of noninfective leafhoppers was numbered and a record was kept of the number on the cage from which the insects were taken. The noninfective leafhoppers in each cage were tested at monthly intervals

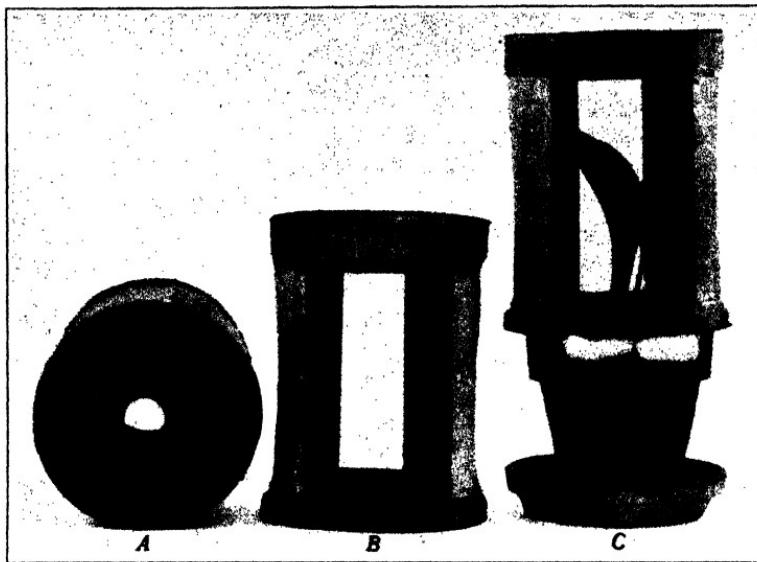


Fig. 1.—View of cages used in determining the period of infectivity during the adult life of the beet leafhopper. *A*, Bottom board with circular hole through which the leaves of a beet seedling projected into the cage; *B*, cage with top and sides covered with lawn, except a glass plate through which observations were made; *C*, cage enclosing a beet seedling with the base of the petioles surrounded with cotton.

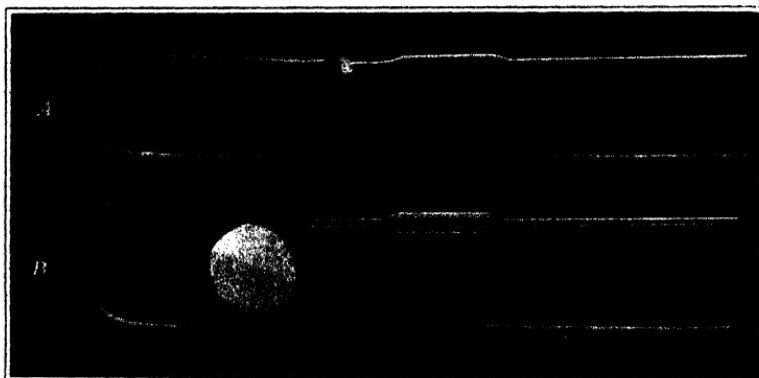


Fig. 2.—Pipettes with a capacity of 10 cc used in capturing beet leafhoppers. *A*, Pipette with a piece of silk bolting covering the opening between the pipette and rubber tube; *B*, pipette, silk bolting, and rubber tube. The end of the rubber tube is held in the mouth. By inhaling a breath of air through the rubber tube, the leafhoppers are drawn into the bulb of the pipette, and by exhaling the breath and snapping the first two fingers against the side of the pipette they are expelled.

by transferring 5 to 10 specimens to a healthy beet seedling. If the non-infective insects in a cage accidentally became infected with curly top, the results with leafhoppers from such a cage were rejected.

Recently molted adults were used in all experiments for two reasons: (1) the insects feed for long periods of time after the last molt and, therefore, more frequently become infective when feeding on a diseased beet, and (2) the age of the adult from the time it acquires the winged stage is known. The long feeding periods may be explained by the fact that before molting the insect stops feeding and empties the contents of the alimentary tract.

To avoid injury, the recently molted adults were allowed to rest for a period of time on the nymphal skins until the wings expanded and the chitin was somewhat hardened. The adults were then captured with a pipette and transferred to an empty cage in which they were confined for a period varying from 2 to 4 hours before feeding them on a curly-top beet.

Each recently molted noninfective adult was transferred from the empty cage with a pipette, gently dropped on a diseased beet leaf, and observed as it fed for short periods. The insect usually inserted its mouth parts into the plant tissue, as could readily be seen by use of a reading glass. Sometimes the leafhopper would withdraw its mouth parts to seek a more suitable place to feed, often on the lower surface of the leaf. In the majority of cases, however, once the insect began to feed, its mouth parts were rarely withdrawn during short feeding periods until interrupted in its meal. During the short feeding periods on curly-top beets a high temperature of approximately 100° F was maintained in the greenhouse. When the leafhopper had fed for the desired length of time, it was interrupted in its meal and transferred to a healthy beet seedling confined in a cage.

Each adult in a cage was transferred at intervals of 24 hours to successive healthy beets. The transfers were made by jarring the cage on the top of the table and by blowing a breath of air through the lawn covering of the cage to cause the leafhopper to hop from the beet seedling to the lawn, wooden frame, or bottom of the cage. The beet was removed by lifting the cage from the clay pot and by pulling the beet leaves through the opening in the bottom. The hand was placed over the opening to prevent the escape of the insect. The cage containing the leafhopper was then placed over another healthy beet.

The beet seedlings, after exposure to the leafhoppers for the desired length of time, were placed in insect-proof cages in the greenhouse. The beets were examined daily for a period of 6 weeks for symptoms of curly

top. If any of the beets developed cleared veinlets on the youngest leaves, the leafhopper had evidently infected the plant during the 24-hour period that it had been confined on the beet.

Repeated capturing of the insects with a pipette was necessary in transferring them from one cage to another in the experiments on the loss of the infective capacity by the leafhoppers when kept on a plant immune to curly top. This resulted in a high mortality, and hence some of the experiments could not be extended over as long a period of time as was desired. In most other experiments in which the leafhoppers were transferred from one plant to another in cages, they were rarely captured by means of the pipette and lived for long periods of time.

The sexes were segregated soon after molting when large numbers of beet leafhoppers were required for an experiment. A large number of last-instar nymphs were usually captured and confined in a cage, and after they passed through the last molt, the male and female leafhoppers were transferred to separate cages. This procedure was followed to prevent fertilization of the females, since eggs deposited by fertilized females would hatch nymphs in the insect-proof cages, which would introduce a source of error into the results.

#### TRANSMISSION OF CURLY TOP BY BEET LEAFHOPPERS FED TEN MINUTES TO THREE HOURS ON A DISEASED BEET

If there is a multiplication of the virus in the insect, those which have fed for short periods of time on curly-top beets should, after the "incubation period" is completed, be able to cause as many infections as those fed for longer periods of time. The following experiment was therefore designed to determine whether a multiplication of the curly-top virus takes place in the leafhopper, or whether the insect only accumulates the virus during repeated feedings and is in reality only an internal mechanical carrier.

Recently molted, previously noninfective leafhoppers were fed for periods of 10, 20, 40, 60, 120, and 180 minutes on a diseased plant and were then transferred to successive healthy beets at intervals of 24 hours during their adult life. If no infections resulted from the feeding of the previously noninfective leafhoppers fed for short periods on a diseased beet during the first 30 days, they were considered noninfective and transfers to successive healthy beets were discontinued. Often, however, after the insects had been discarded, one or more of the beets inoculated by such specimens would develop symptoms of the disease, which indicated that the insects had been infective.

Severin<sup>(1)</sup> has demonstrated that noninfective beet leafhoppers were able to recover the curly-top virus from beet seedlings with from 4 to 6 leaves 2 days after infective insects were first fed on them. The cleared veinlets sometimes appeared on the youngest leaves at the end of 2 days, but noninfective insects did not always become infective by feeding on seedlings showing this early symptom. These results indicate that leafhoppers can recover virus by feeding for several days on plants which they have infected. This fact has not always been considered in transmission experiments in which an attempt was being made to deter-

TABLE 1

INFECTION OF BEET LEAFHOPPERS WITH CURLY TOP BY FEEDING  
FOR SHORT PERIODS ON DISEASED BEETS

Period on curly-top beet, minutes	Number fed on curly-top beets	Number infective	Number not infective	Per cent infective
10.....	16	7	9	43.7
20.....	18	7	11	38.9
40.....	11	7	4	63.6
60.....	11	7	4	63.6
120.....	6	3	3	50.0
180.....	13	7	6	53.9
Total.....	75	38	37	....
Average.....	...	...	...	50.8

mine how long the insect vector retained the infective capacity. The feeding period of the leafhoppers in the present experiments was only 24 hours on each beet, and it was considered impossible for them to recover any virus which they had inoculated into and which had multiplied in the beet during the period they were confined on the seedling.

Seventy-five recently molted, previously noninfective leafhoppers were fed for short periods on diseased beets, as shown in table 1, and of this number 38 acquired and transmitted the virus to beets, while 37 failed to become infective. The results in table 1 suggest that there was a slight increase in the percentage that acquired the infective power as the length of the feeding periods was increased. The data indicate that 50.8 per cent of the insects allowed to feed for short periods on a diseased beet became infective.

The results in table 2 show a great variation in the delay in the development of the infective capacity in single beet leafhoppers. The period of time that elapsed between the initial feeding on a curly-top beet and the first infection by the leafhopper varied from 1 to 44 days, or an average of 9.6 days when the insects were kept at temperatures varying

TRANSMISSION OF CURLY TOP BY SINGLE BEET LEAFHOPPERS FED FROM 10 TO 180 MINUTES ON A DISEASED BEET AND ON A HEALTHY BEET DAILY DURING ADULT LIFE

Period on curly-top beet, minutes	Sex	No.	Dates fed on healthy beets	Beets infected	Beets inocu- lated	Per cent	Period of development of infective capacity, days	Adult age when last infection produced, days	Longest period between two in- fections, days	Per cent of total infections during				
										Period between last infection and death of insect, days	1-30 days			
											31-60 days	61-90 days	91-120 days	
10	♀	1	July 8-Aug. 15	39	1	2.6	36	...	3	0.0	100.0	...	...	
		2	Sept. 7-Oct. 19	49	1	2.0	44	44	5	0.0	100.0	...	...	
		3	Sept. 4-Nov. 19	77	2	2.6	50	35	27	50.0	30.0	0.0	...	
	♂	4	May 8-July 30	83	4	4.8	9	32	12	51	75.0	25.0	0.0	...
20	♀	1	June 25-Aug. 19	55	4	7.3	1	31	21	24	75.0	25.0	...	...
		2	Sept. 3-Dec. 20	108	3	2.8	5	81	43	27	33.3	33.3	0.0	...
		5	July 9-Aug. 28	52	3	5.8	7	45	35	7	33.3	66.7	...	...
	♂	6	Sept. 6-Mar. 9	183	1	0.5	1	1	...	182	100.0	0.0	0.0	0.0
40	♀	3	June 25-Aug. 8	44	1	2.3	15	15	...	29	100.0	0.0	...	...
		4	June 24-Oct. 27	95	6	6.3	1	71	24	24	83.3	0.0	16.7	0.0
		5	June 3-Oct. 29	150	2	1.3	7	117	110	33	50.0	0.0	50.0	0.0
	♂	7	July 7-Sept. 3	58	1	1.7	7	7	...	51	100.0	0.0	0.0	...
120	♀	8	June 3-Dec. 5	184	4	2.2	10	161	105	23	25.0	0.0	0.0	25.0
		6	May 7-July 28	83	5	6.0	6	34	10	49	80.0	20.0	0.0	...
		7	June 25-Nov. 4	132	2	1.5	8	17	8	115	100.0	0.0	0.0	...
	♂	9	May 25-July 25	61	3	4.9	10	18	10	43	100.0	0.0	0.0	...
180	♀	10	July 7-Oct. 27	111	3	2.7	7	18	7	93	100.0	0.0	0.0	...
		11	May 7-Aug. 18	103	2	1.9	4	21	16	82	100.0	0.0	0.0	...
		12	July 7-Jan. 4	180	7	3.9	7	130	77	50	42.9	0.0	0.0	28.6
	♂	8	June 3-Nov. 10	160	5	3.1	8	68	45	92	80.0	0.0	20.0	0.0
Average	♀	13	May 7-Sept. 1	117	6	5.1	9	77	25	40	33.3	33.3	0.0	...
		9	June 3-July 28	55	5	9.1	3	25	9	30	100.0	0.0	0.0	...
		10	June 25-Sept. 15	82	1	1.2	1	1	...	81	100.0	0.0	0.0	...
	♂	11	May 8-Aug. 14	98	3	3.1	9	32	21	66	66.7	33.3	0.0	0.0
Average	♂	12	Sept. 6-Jan. 4	121	9	7.4	9	105	38	16	44.4	33.3	11.1	11.1
		...	...	99.2	34	3.4	9.6	49.5	34.3	49.7	7.8*	8.9*	1.6*	1.0*

\* Per cent beets infected.

from 70° to 100° F in the greenhouse. The leafhoppers caused infections at very irregular intervals after the first infection.

The leafhoppers fed for short periods of from 10 to 180 minutes on a curly-top beet were able to cause only a few infections. As shown in

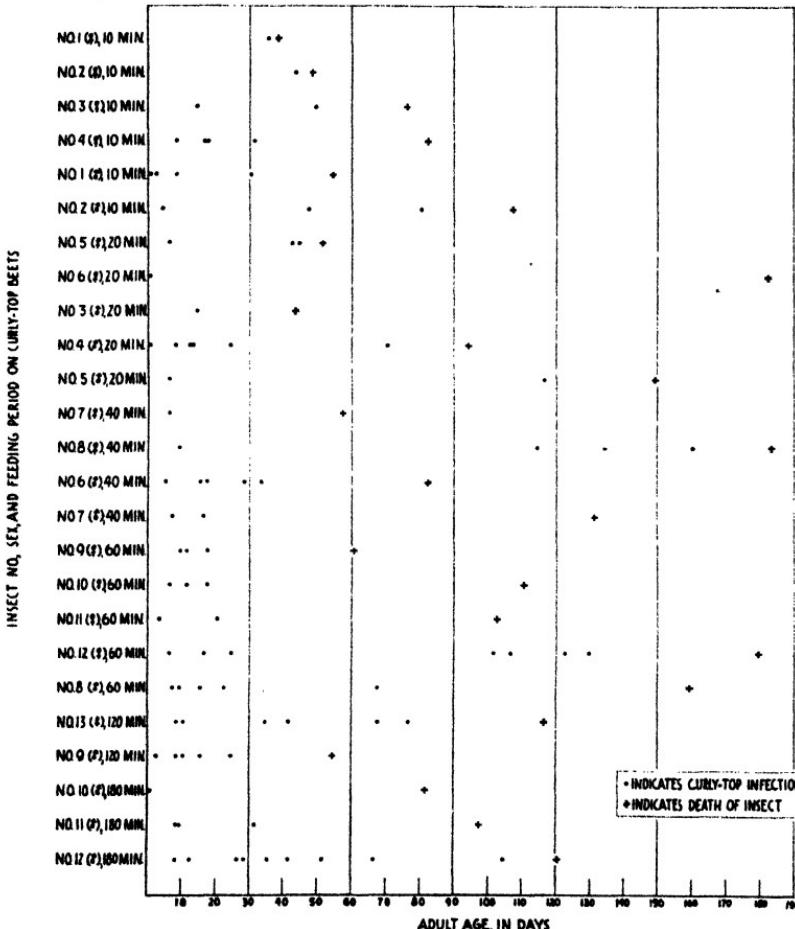


Fig. 3.—Number and distribution of curly-top infections produced by single beet leafhoppers fed for short periods varying from 10 to 180 minutes on diseased beets and transferred daily to a healthy beet. The insects produced most of the infections during the first 30 days of adult life, after which there was a decrease in the infective capacity of the insects.

Table 2, six leafhoppers fed for a period of 10 minutes on a diseased beet infected from 1 to 4 beets, five fed 20 minutes infected from 1 to 6 beets, four fed 40 minutes infected from 1 to 5 beets, five fed 60 minutes infected from 2 to 7 beets, two fed 120 minutes infected from 5 to 6 beets,

and three fed 180 minutes infected from 1 to 9 beets during adult life. The insects fed for short periods on curly-top beets averaged 3.4 infections when each was provided with a healthy beet seedling daily during its adult life.

The data in table 2 indicate that the leafhoppers were slightly infective throughout life, but that they were highly infective only during the first 30 days of adult life. The age of the adults when the last infection was produced varied from 1 to 161 days, and averaged 49.5 days. The number of beets inoculated, which also represents the longevity of the leafhoppers in days, varied from 39 to 184 and averaged 99.2. The maximum period between any two infections varied from 7 to 110 days and averaged 34.3 days, while the number of days between the last infection and the death of the insect varied from 3 to 182 days and averaged 49.7 days. The data show an average of 7.2 per cent of the beets were infected during the first 30 days of adult life. There was a marked decline in the number of infections during the second 30-day period to an average of 2.2 per cent and a further decrease during the third 30-day period to an average of 1.3 per cent. After these periods the percentage of beets infected remained approximately constant until the death of the insect.

Figure 3 shows the frequency and distribution of curly-top transmissions by beet leafhoppers fed for short periods on diseased beets and indicates that a majority of the infections are caused during the first 30 days of adult life. The results indicate that after this period the frequency of curly-top infections by the leafhopper becomes less until the ninetieth day, after which the frequency of infections remains approximately constant. After the period of delay in the development of the infective capacity, there are no indications of any cycles of development nor any evidence that the virus multiplies in the insect vector. Some leafhoppers apparently retained the infective capacity during their entire adult life, as shown by female No. 8 and male No. 12. Others, such as female No. 6 and male No. 8 apparently lost the power to cause infection during later life, as shown in figure 3.

#### TRANSMISSION OF CURLY TOP BY BEET LEAFHOPPERS FED FROM SIX HOURS TO TWENTY-EIGHT DAYS ON DISEASED BEETS

In order to determine whether there was any direct relation between the length of time a beet leafhopper feeds on a diseased plant and the number of infections it produces during its adult life, 18 recently molted previously noninfective leafhoppers were fed for periods of  $\frac{1}{4}$  (6 hours),

$\frac{1}{2}$  (12 hours), 1, 3, 7, 14, 21, and 28 days on two curly-top beets, 10 males on one and 8 females on the other. Each insect was then provided daily with successive healthy beets.

The results of the experiment show a great variation in the number of infections produced by the beet leafhoppers fed for different periods of

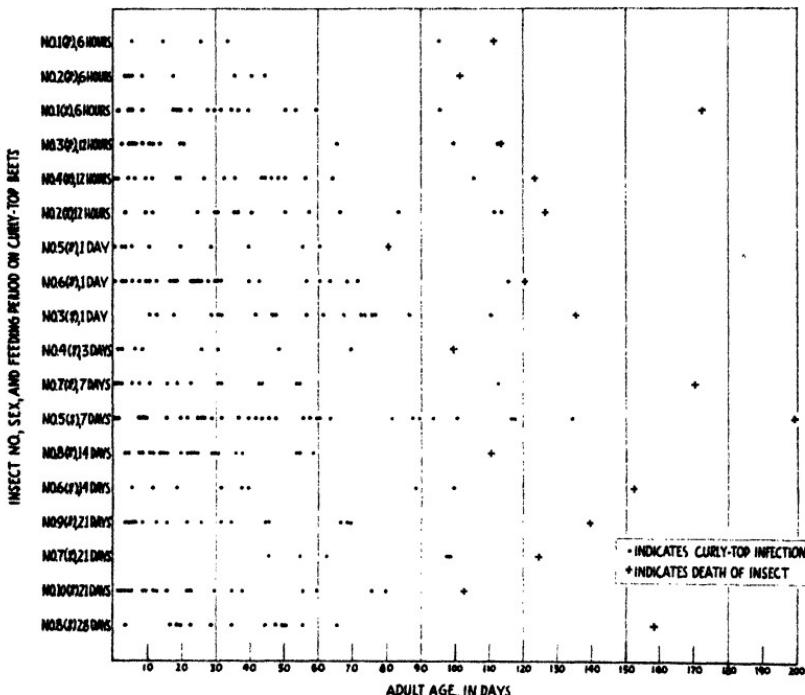


Fig. 4.—Number and distribution of curly-top infections produced by single beet leafhoppers fed from 6 hours to 28 days on a diseased beet and transferred daily to a healthy beet. Most of the infections occurred during the first 30 days of adult life. The infective power was apparently lost by some of the leafhoppers toward the end of their natural life, but others retained the infectivity up to the time of death.

time on curly-top beets. The number of beets infected by insects fed from 6 hours to 28 days on a curly-top beet varied from 5 to 32, or an average of 15.1, as shown in table 3. One specimen fed for 6 hours and another for 21 days on a curly-top beet produced only 5 infections, while a third insect fed for 7 days infected 32 beets during its adult life, as indicated in table 3. From 4.0 to 22.3 per cent, or an average of 11.6 per cent, of the beets were infected with curly top during the adult life of the leafhopper.

The data in table 3 also indicate that in the majority of the beet leaf-hoppers there was a decrease in the infective capacity during the later

TABLE 3  
TRANSMISSION OF CURLY TOP BY SINGLE BEET LEAFHOPPERS FED FROM 6 HOURS TO 28 DAYS ON A DISEASED BEET AND ON A HEALTHY BEET DAILY DURING ADULT LIFE

Period on curly-top beet, days	Sex	No.	Dates fed on healthy beets	Beets inoculated	Beets infected	Period of development of infective capacity, days	Longest delay in development of infective capacity, days	Longest period between last infection produced, days	Per cent of total infections during				
									1-30 days	31-60 days	61-90 days	91-120 days	121-150 days
1	♂	1	May 3-Aug. 22	112	5	4.5	6	96	62	60.0	20.0	0.0	20.0
		2	May 3-Aug. 12	102	8	7.8	4	45	57	62.5	37.5	0.0	0.0
1	♀	1	May 3-Oct. 22	173	18	10.5	2	96	36	77	55.5	38.9	0.0
		2	May 3-Aug. 24	114	13	11.4	3	113	45	1	76.9	0.0	7.7
1	♂	3	May 3-Aug. 24	124	19	15.3	1	106	41	18	47.4	42.1	5.3
		4	May 3-Sept. 3	127	15	11.8	4	114	28	13	33.3	40.0	13.3
1	♀	2	May 3-Sept. 6	127	15	11.8	4	114	28	13	33.3	40.0	13.3
		3	May 4-July 23	91	10	12.3	1	61	16	20	70.0	20.0	10.0
1	♂	5	May 4-Sept. 1	121	27	22.3	1	116	44	5	63.0	18.5	14.9
		6	May 4-Sept. 16	136	18	13.2	11	111	24	25	22.2	33.3	36.9
3	♀	4	May 6-Aug. 13	100	8	8.0	2	70	21	30	62.5	25.0	12.5
		5	May 10-Oct. 27	171	16	9.4	1	113	58	58	56.3	37.5	0.0
7	♂	7	May 10-Nov. 25	200	32	16.0	1	135	18	65	37.5	31.3	16.6
		9	May 10-Nov. 25	153	8	5.2	6	100	48	53	37.5	37.5	25.0
14	♂	8	May 17-Sept. 4	111	22	19.8	4	59	16	52	72.7	27.2	0.0
		6	May 17-Oct. 15	153	8	5.2	6	100	48	53	37.5	37.5	0.0
21	♂	9	May 24-Oct. 10	140	16	11.4	4	70	21	70	56.3	25.0	18.7
		7	May 24-Sept. 25	125	5	4.0	46	99	35	26	0	40.0	40.0
28	♂	10	May 31-Sept. 10	103	19	18.4	2	80	18	23	68.4	21.1	10.5
		8	May 31-Oct. 5	159	13	8.2	4	66	13	93	46.1	46.1	7.7
Average	..	..	.....	130.7	15.1	11.6	5.7	91.7	31.2	39	86.5*	15.0*	6.6*
												3.9*	0.5*

\* Per cent beets infected.

stages of adult life. The age of the adults when the last infection was produced varied from 45 to 135 days, or an average of 91.7 days, while the longevity of the adults varied from 81 to 200 days, or an average of 130.7 days. As already stated, each insect was provided with a healthy beet daily during adult life, and hence the longevity of the adults in days corresponds to the number of beets inoculated in table 3. The longest period between two successive infections varied from 13 to 62 days, or an average of 31.2 days, while the period between the last infection and the death of the insect varied from 1 to 93 days, or an average of 39 days. In 11 of 18 specimens tested, the longest period between successive infections was less than the period between the last infection and the death of the insect; this fact suggests a decrease in the infective capacity of the insect.

The majority of leafhoppers produced more infections during the first 30 days of their adult life than during any succeeding 30-day period, as shown in figure 4. The percentage of the total beets infected during successive 30-day periods of adult life indicates a progressive decrease in the infective capacity, which suggests that the quantity of virus in the insect is being decreased as the insect feeds on consecutive healthy beets. The average percentages of beets infected during successive 30-day periods were as follows: 26.5, 15.0, 5.6, 3.9, and 0.5 respectively (table 3).

#### TRANSMISSION OF CURLY TOP BY BEET LEAFHOPPERS FED DURING NYMPHAL STAGES ON DISEASED BEETS

In connection with the preceding experiments on short feeding periods, it became desirable to determine if the length of feeding period on a source of virus would affect the number of infections produced. Accordingly, 10 recently molted adults which had fed on diseased beets during their entire nymphal stages were provided with successive healthy beets at 24-hour intervals during their adult life.

There was a great irregularity in the number of infections produced by the infective adults. These leafhoppers infected from 4 to 26 beets, or an average of 15.6 beets, during their adult life, as shown in table 4. They infected from 3.5 to 44.1 per cent, or an average of 11.8 per cent, of the beets on which they fed.

The age of the adults when they produced the last infection varied from 50 to 151 days, or an average of 81.3 days, while the number of beets inoculated, which also represents the longevity of the adults in days, varied from 58 to 206, or an average of 132.2. The longest period between two successive infections produced by a single specimen varied

TABLE 4  
TRANSMISSION OF CURLY TOP BY INFECTIVE BEET LEAFHOPPERS FED DURING NYMPHAL STAGES ON A HEALTHY BEET DAILY DURING ADULT LIFE

Sex	No.	Dates fed on healthy beets	Beets inoculated	Beets infected		Adult age when last infection produced: days	Longest period between two infections: days	Period between last infection and death of insect: days	Per cent of total infections during			
				Number	Per cent				31-60 days	61-90 days	91-120 days	121-150 days
♀	1	Sept. 4-Oct. 31	88	10	17.2	50	13	8	80.0	20.0	4.5	...
	2	Sept. 4-Nov. 26	83	22	26.5	83	27	0	54.5	40.9	4.5	...
	3	Sept. 4-Nov. 28	87	11	12.6	59	16	28	63.6	36.4	0.0	...
	4	May 26-Sept. 15	113	4	3.5	90	47	33	50.0	25.0	0.0	...
	5	Aug. 24-Dec. 20	119	12	10.1	87	16	32	50.0	25.0	0.0	...
	6	Sept. 4-Jan. 25	144	22	15.3	67	20	77	72.7	16.2	9.1	0.0
	7	June 26-Dec. 11	168	18	10.7	50	12	118	83.3	16.7	0.0	0.0
	8	May 7-Oct. 27	173	17	9.8	108	25	65	47.1	11.8	35.3	0.0
	9	June 25-Dec. 31	169	15	7.9	104	42	85	26.7	60.0	0.0	13.3
	10	Sept. 4-Mar. 28	206	9	3.9	151	33	55	75.0	0.0	12.5	0.0
♂	1	May 26-July 24	59	26	44.1	59	7	0	61.5	38.5	...	...
	2	Aug. 24-Jan. 28	168	22	11.7	78	26	110	68.2	31.8	0.0	0.0
Average	.....	.....	132.2	15.6	11.8	81.3	27.7	50.9	51.9*	14.8*	1.2*	0.0*

\* Per cent beets infected.

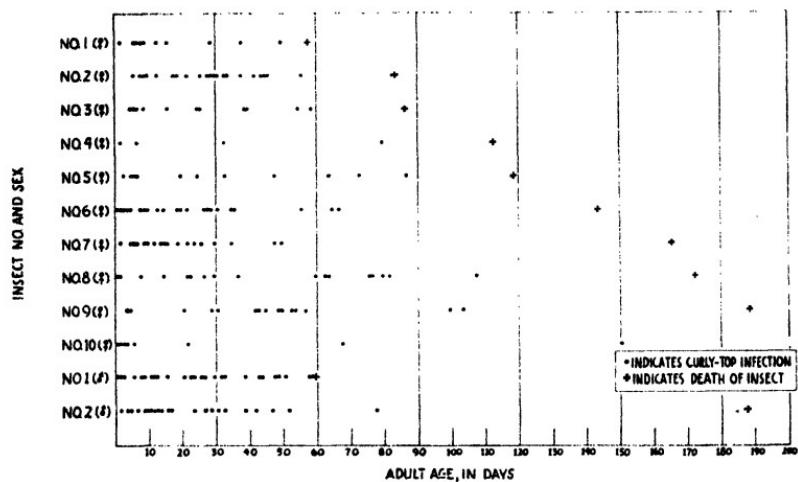


Fig. 5.—Frequency and distribution of curly-top infections by single beet leafhoppers that had completed the nymphal stages on diseased beets and were transferred daily to healthy beets during adult life. Some of the leafhoppers apparently lost the infective power during late adult life. Most of the infections were caused during early adult life.

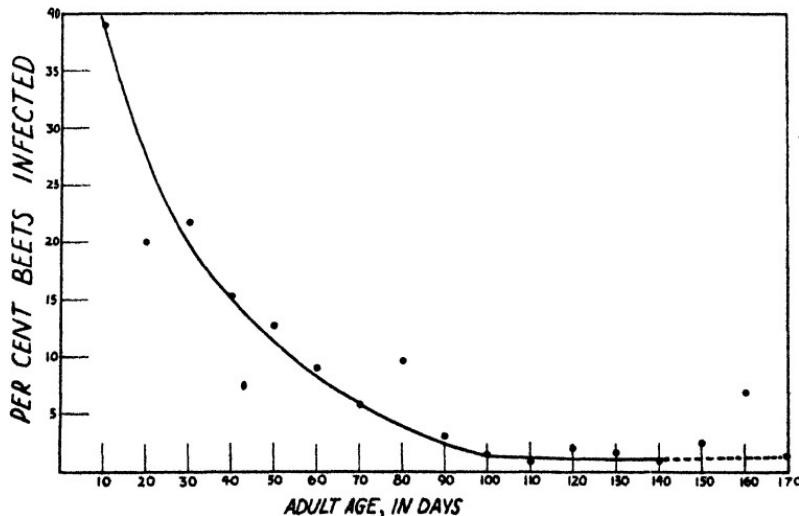


Fig. 6.—Decrease in infective capacity of the beet leafhopper as indicated by percentage of beets infected during successive 10-day periods of adult life. The nymphal stages were completed on curly-top beets and the adults were provided daily with a healthy beet until the end of their natural life.

from 7 to 83 days, or an average of 27.7 days, while the period between the last infection and the death of the insect varied from 0 to 118 days, or an average of 50.9 days (table 4). These results indicate a decrease in the power of the leafhoppers to cause infection as they approach old age.

As in preceding experiments with adult beet leafhoppers fed for varying periods on a curly-top beet, the insects bred as nymphs on curly-top beets also infected more beets during the first 30 days of their adult life than during any succeeding 30-day period. The average percentages of beets infected during successive 30-day periods of adult life (table 4) were as follows: 31.9, 14.8, 5.2, 1.2, 0.0, 0.8. These data indicate a gradual decrease of the infective capacity. The decrease and the fact that most beets are infected during early adult life of the insect are shown in figure 5, which gives the distribution of the infections caused by each beet leafhopper. Most of the beets were infected during early adult life, and as the insects grew older the frequency of the infections became less until finally most leafhoppers were no longer capable of causing infection. Several of the specimens failed to infect a beet for a long period of time and then suddenly produced several infections, as shown in figure 5 by female No. 9, but this was not usually the case. The leafhopper did not produce the disease in any characteristic cycles of infectivity, such as might be expected if the virus multiplied or passed through some sort of a cycle of development in the insect.

The gradual decrease of the infective capacity of the beet leafhopper is also illustrated by the curve in figure 6, which shows the percentage of beets on which the insects fed that were infected during successive 10-day periods of adult life.

#### TRANSMISSION OF CURLY TOP BY MALE AND FEMALE LEAFHOPPERS

A comparison was made of the transmission of curly top by male and female beet leafhoppers to determine if one of the sexes might not be a more efficient vector of the virus than another. If one sex proved more efficient in the transmission of the disease, better results would be obtained from experiments which were carried out with such individuals.

A lot of 8 male and 8 female leafhoppers that had completed the nymphal stages on the same diseased beet were each transferred to a healthy beet soon after completing the last molt. The single insects were transferred to successive healthy beets at intervals of 24 hours during the remainder of their adult life.

As shown in table 5, the males infected from 5 to 50 beets, or an aver-

TABLE 5  
TRANSMISSION OF CURLY TOP BY SINGLE BEET LEAFHOPPERS FED DURING NYMPHAL STAGES ON A DISEASED BEET AND ON A HEALTHY BEET DAILY DURING ADULT LIFE

Sex	No.	Beets inoculated	Beets infected	Beets inoculated	Beets infected	Adult age when last infection produced, days	Period between last infection and death of insect, days	Per cent of total infections during									
								Sept. 19-Feb. 18	10	6.5	123	52	29	50.0	20.0	0.0	10.0
♂	1	Sept. 19-Feb. 18	163	10	6.5	123	52	29	50.0	20.0	0.0	10.0	0.0	10.0	0.0	10.0	0.0
	2	Sept. 19-Dec. 27	100	12	12.0	94	18	16	55.3	25.0	16.7	0.0	0.0	0.0	0.0	0.0	0.0
	3	Sept. 19-Feb. 10	146	15	10.3	75	20	70	40.0	46.7	13.3	0.0	0.0	0.0	0.0	0.0	0.0
	4	Sept. 19-Feb. 25	160	5	3.1	125	60	23	40.0	30.0	30.0	0.0	0.0	0.0	0.0	0.0	0.0
	5	Sept. 18-Dec. 4	77	12	15.6	77	16	0	50.0	33.3	17.6	0.0	0.0	0.0	0.0	0.0	0.0
	6	Sept. 18-Apr. 15	209	15	7.2	148	73	61	66.7	6.7	30.0	0.0	0.0	0.0	0.0	0.0	0.0
	7	Sept. 18-Apr. 7	201	50	24.9	158	21	43	42.0	30.0	22.2	4.0	6.0	6.0	6.0	6.0	6.0
	8	Sept. 18-Nov. 21	64	14	21.9	56	24	8	78.6	31.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Av.		.....	138.6	16.6	12.0	105.7	35.5	31.2	28.5*	12.9*	11.4*	1.2*	4.1*	4.1*	4.1*	4.1*	4.1*
♀	1	Sept. 19-Apr. 5	199	18	9.0	164	35	35	38.9	22.2	16.7	5.5	5.5	11.1	11.1	11.1	11.1
	2	Sept. 19-Jan. 6	110	5	4.5	62	36	48	50.0	30.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	Sept. 19-Feb. 14	149	12	8.0	146	70	3	41.7	33.3	16.7	0.0	0.0	0.0	0.0	0.0	0.0
	4	Sept. 19-Mar. 31	194	15	7.7	75	23	116	60.0	33.3	6.7	0.0	0.0	0.0	0.0	0.0	0.0
	5	Sept. 18-Mar. 27	190	15	7.9	167	69	23	53.3	20.0	0.0	6.7	6.7	13.3	13.3	13.3	13.3
	6	Sept. 18-Mar. 8	171	3	1.7	63	46	108	33.3	33.3	33.3	0.0	0.0	0.0	0.0	0.0	0.0
	7	Sept. 18-Feb. 26	161	13	8.1	150	49	2	46.2	15.2	0.0	15.2	0.0	0.0	0.0	0.0	0.0
	8	Sept. 18-Jan. 6	109	5	4.6	10	4	99	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Av.		.....	160.4	10.7	6.7	106.1	41.9	54.2	18.7*	7.8*	7.7*	1.7*	1.7*	1.7*	1.7*	1.7*	1.7*

\* Per cent beets infected.

age of 16.6 beets, during their adult life. The females infected from 3 to 18 beets, or an average of 10.7 beets, during their adult life. The males infected an average of 12.0 per cent, while the females infected an average of 6.7 per cent of the beets on which they fed. The results of this experiment seem to indicate that the males have a slightly greater ability

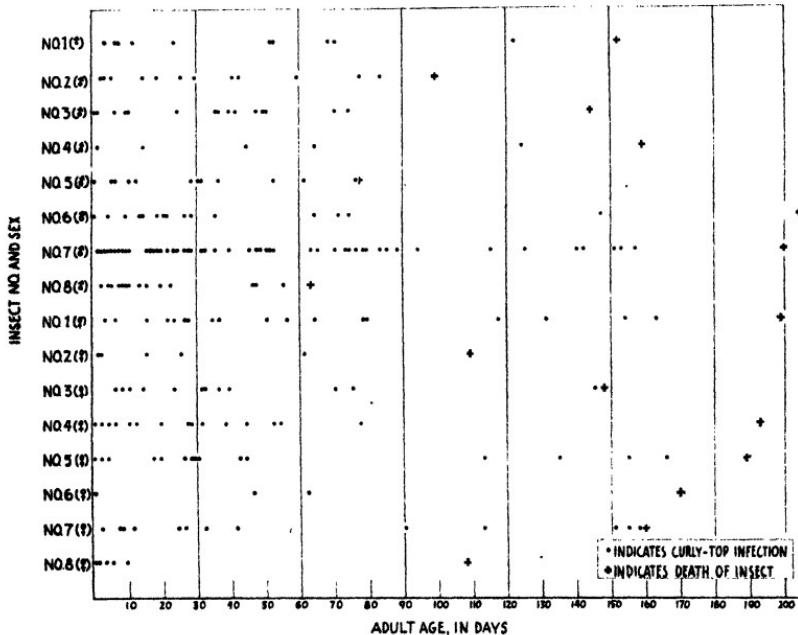


Fig. 7.—Number and distribution of curly-top infections produced by 8 male and 8 female beet leafhoppers which had completed the nymphal stages on the same diseased beet and were transferred daily to healthy beets during their adult life. Most of the infections occurred during the first 30 days of adult life, and, while some insects apparently lost the infective capacity as they approached old age, others retained the power to cause infection.

to transmit the virus. The great variation in the number of beets infected by single male and female adults, however, precludes any definite conclusion.

The results in table 5 indicate that the two sexes retained the power to transmit the virus for approximately the same length of time. The age of the males when they produced the last infection averaged 105.7 days, while the age of the females averaged 106.1 days when they produced the last infection. The longevity (corresponding with the number of beets inoculated) of the females in days was greater than that of the males, averaging 160.4, while the males averaged 138.6 (table 5).

The distribution of the beets infected during the adult life of the two

sexes was very similar. The males infected an average of 28.3 per cent and the females 18.7 per cent of the beets they fed on during the first 30 days of adult life (table 5). During the period from 31 to 60 days, the males infected an average of 12.9 per cent and the females an average of 7.9 per cent of the beets. After a continued decrease during the next 30-day period, as shown in table 5, the average infective capacity of the two sexes remained approximately constant.

Figure 7 illustrates the distribution of infections by the eight male and eight female adults and shows that most of the infections were produced during early adult life. Some of the insects, for example, male No. 3 and females No. 4 and No. 8, failed to infect beets toward the end of their natural life; this indicates that they had apparently lost the infective capacity, while other specimens, such as females No. 3 and No. 7 retained their infectivity up to the time of their death.

#### LOSS OF INFECTIVE CAPACITY BY LEAFHOPPERS KEPT ON PLANT IMMUNE TO CURLY TOP

Experiments were conducted to determine whether infective beet leafhoppers would lose the infective power when kept on a plant immune to curly top, such as Alameda or Mammoth sweet corn (*Zea mays*). In three different experiments, one hundred recently molted adults which had completed their nymphal stages on a diseased beet were transferred singly to healthy beets for a period of 24 hours to determine the percentage of insects that would transmit curly top during that period. The leafhoppers were fed on sweet corn for a period of 9 days, and then on the 10th day they were transferred singly to healthy beet seedlings for 24 hours. The procedure was repeated several times up to the 90th day of the adult life, when the number of insects was reduced so low that further tests were impossible owing to the high mortality caused by the repeated capture of the insects with the pipette.

The results of the three experiments indicate a gradual loss of the infective capacity by the leafhopper when confined on sweet corn. Table 6 summarizes the results of the three experiments and shows that 50.8 per cent of the insects transmitted curly top during the first transfer to healthy beets. On the 10th day 55.5 per cent of the healthy beets became diseased, after which time, with succeeding 10-day periods, there was a drop in the percentage of leafhoppers that transmitted curly top, until the 80th day when only 3 per cent, and on the 90th day 3.1 per cent, were still able to cause infection. The data obtained in these three experiments are plotted in figure 8, which shows the loss of the infective capacity by the leafhoppers when confined on sweet corn.

TABLE 6

LOSS OF INFECTIVE CAPACITY BY BEET LEAFHOPPERS KEPT ON SWEET CORN  
AND TRANSFERRED EVERY TENTH DAY TO A HEALTHY BEET

Days on sweet corn	Females		Males		Per cent infected
	Beets inoculated	Beets infected	Beets inoculated	Beets infected	
Experiment No. 1					
1	50	30	50	29	59.0
10	50	31	50	36	67.0
20	50	17	50	26	43.0
30	50	9	50	19	28.0
40	65	24	35	14	38.0
50	42	4	13	1	9.1
60	29	0	3	2	6.6
70	21	4	..	..	19.0
80	16	1	..	..	6.3
90	..	..	..	..	..
Experiment No. 2					
1	50	20	50	18	38.0
10	25	15	25	12	54.0
20	25	8	25	15	46.0
30	25	4	25	9	26.0
40	25	5	25	3	16.0
50	23	0	25	1	2.1
60	13	1	22	3	11.0
70	7	2	7	1	21.0
80	..	..	..	..	..
90	..	..	..	..	..
Experiment No. 3					
1	..	..	50	30	60.0
10	..	..	50	17	34.0
20	..	..	50	17	34.0
30	..	..	50	8	16.0
40	..	..	50	9	18.0
50	..	..	50	8	16.0
60	50	2	11	0	3.3
70	50	4	..	..	8.0
80	50	1	..	..	2.0
90	32	1	..	..	3.1
Results of 3 experiments summarized					
1	100	50	150	77	50.8
10	75	46	125	65	55.5
20	75	25	125	58	41.5
30	75	13	125	36	24.5
40	90	29	110	26	27.5
50	65	4	88	10	9.2
60	92	3	36	5	6.3
70	78	10	7	1	12.0
80	66	2	..	..	3.0
90	32	1	..	..	3.1

The results of the three preceding experiments demonstrate that the beet leafhopper gradually loses its infective capacity when not given the opportunity to become reinfected by feeding on a curly-top beet. The leafhopper may gradually exhaust its store of virus by giving off a certain quantity of virus in the saliva during feeding.

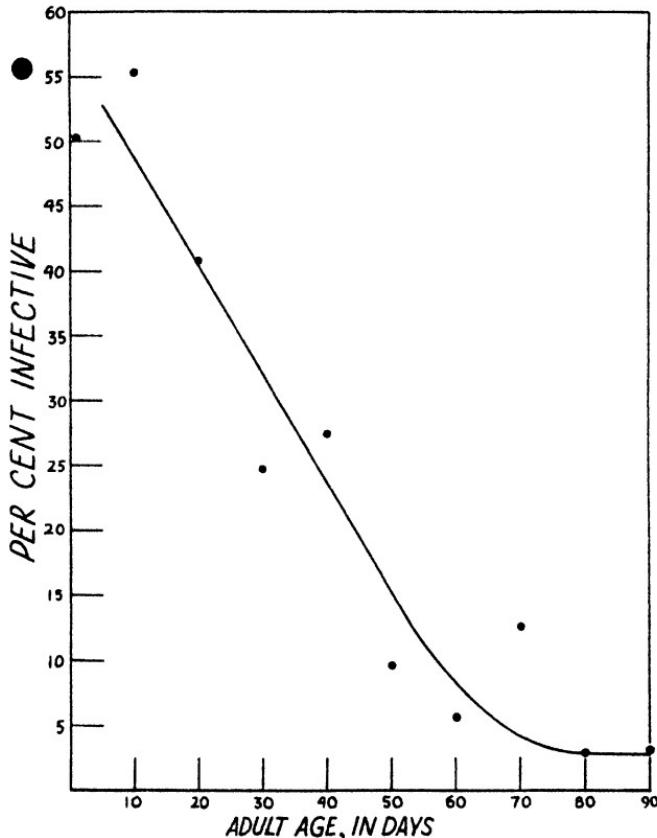


Fig. 8.—Percentage of beet leafhoppers which were infective during successive 10-day periods of adult life. The nymphal stages were completed on diseased beets and the adults were fed successively on sweet corn for 9 days and on healthy beets for 1 day during their entire adult life.

#### ABILITY OF BEET LEAFHOPPER TO ACQUIRE AND TRANSMIT CURLY TOP DURING ADULT LIFE

Two explanations were considered possible for the fact that the infective beet leafhopper caused fewer infections as it approached old age than during early adult life. The insect may gradually lose the ability to

transmit the curly-top virus because of physiological changes brought about by old age, or it may simply be depleting its store of virus in feeding as it is transferred daily to a healthy beet. Experiments were performed to determine the ability of the beet leafhopper to transmit curly top during various periods of adult life.

Previously noninfective beet leafhoppers which had spent 10, 30, 40, 60, 70, 75, 90, 100, and 120 days of their adult life on sweet corn were tested for their ability to acquire and transmit curly top. In each test 20 leafhoppers were fed for one day on a curly-top beet and were then

TABLE 7

ABILITY OF BEET LEAFHOPPERS TO ACQUIRE AND TRANSMIT CURLY TOP DURING  
VARIOUS PERIODS OF ADULT LIFE\*

Adult age, in days	Adults infective		Adult age, in days	Adults infective		Adult age, in days	Adults infective	
	Number	Per cent		Number	Per cent		Number	Per cent
Experiment No. 1			Experiment No. 2			Experiment No. 3		
10.....	12	60	10.....	13	65	10.....	11	55
40.....	14	70	30.....	7	35	30.....	11	55
70.....	10	50	60.....	6	30	60.....	5	25
100.....	9	45	90.....	9	45	75.....	9	45
						100.....	2	10
						120.....	7	35

\* The twenty leafhoppers used in each test were fed for 1 day on a curly-top beet and 10 days on a healthy beet.

transferred singly to 20 healthy beets on which they were kept for a period of 10 days. The results of three experiments are presented in table 7.

The data in table 7 show the percentage of leafhoppers which were able to acquire and transmit curly top during various periods of adult life to be as follows: Experiment No. 1, 60 per cent on the 10th day and 45 per cent on the 100th day, experiment No. 2, 65 per cent on the 10th day and 45 per cent on the 90th day, and experiment No. 3, 55 per cent on the 10th day and 35 per cent on the 120th day. These percentages show losses of only 15 to 20 per cent in the ability of leafhoppers to acquire and transmit curly top during later adult life as compared with early adult life. Figure 9 shows the results of the three experiments. This loss is not great enough to explain the loss of the infective capacity by leafhoppers which have not had access to a source of virus for a long period of time.

Figure 10 shows that only 10 to 21 per cent of the leafhoppers fed

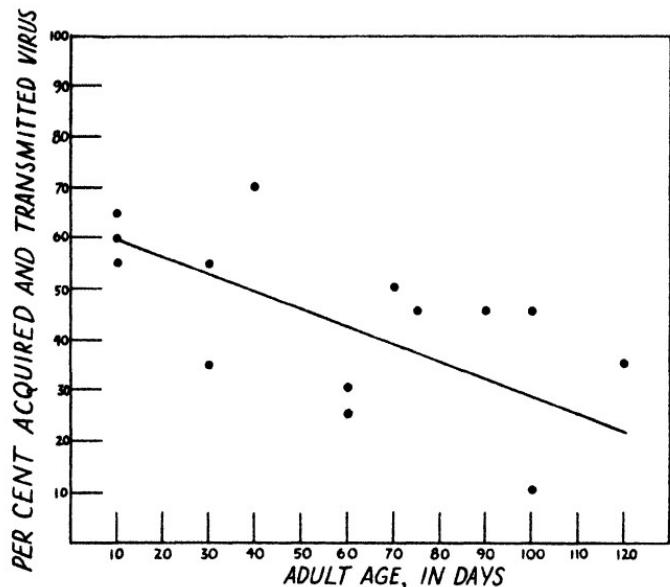


Fig. 9.—Percentage of noninfective beet leafhoppers kept on sweet corn and tested at different ages that acquired the virus by feeding 1 day on a curly-top beet and transmitted it to a healthy beet during the following 10 days.

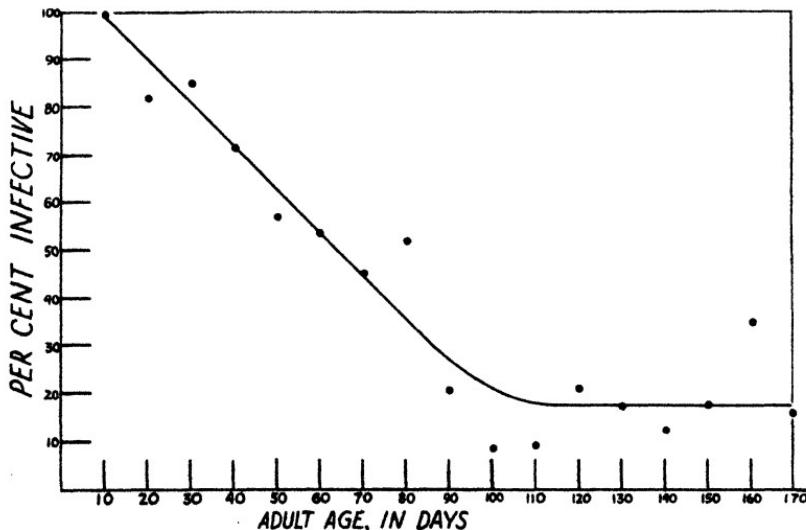


Fig. 10.—Decrease of infective capacity in beet leafhoppers as indicated by the percentage of them which infected beets during successive 10-day periods of adult life. The nymphal stages were completed on diseased beets and the adults were fed daily on a healthy beet during their natural life. (Data from tables 4 and 5.)

during the nymphal stages on diseased beets were still infective from the 100th to 120th day of adult life, as indicated by the percentage of them which infected beets during 10-day periods, when transferred daily to a healthy beet. Since 100 per cent of these insects were infective during the first ten days of adult life, the percentages show a decrease in the infective capacity of 79–90 per cent, which is several times greater than that shown in figure 9 for the loss of the ability to acquire and transmit curly top by previously noninfective leafhoppers. The data in figure 10 were taken from tables 4 and 5.

These data present additional evidence in favor of the theory that infective beet leafhoppers slowly decrease their store of virus and that if they are not reinfected by feeding on a diseased plant to replenish their store of virus, the infective power is gradually lost. Probably the marked decrease in the number of infections by the leafhoppers was only slightly due to physiological changes brought about by the aging of the insect.

#### REINFECTION OF BEET LEAFHOPPERS WITH CURLY TOP DURING LATER ADULT LIFE

Previous experiments showed that many of the leafhoppers lost the capacity to produce infection during the latter part of their adult life when they were transferred daily to a healthy beet. Two experiments were conducted to determine whether this was due to an exhaustion of the insect's supply of virus or whether the insects had developed an immunity to the virus after having once been infective. Immunity would be indicated by a failure to become reinfected after having once been infective and then having become noninfective.

In the first experiment four recently molted infective leafhoppers that had completed their nymphal stages on a diseased beet were transferred to sweet corn for a period of 40 days, and then each insect was provided with a healthy beet at intervals of 24 hours for 10 days. They were then returned to sweet corn for a period of 43 days, after which they were again transferred daily to successive healthy beets for a period of 53 days. The leafhoppers were reinfected with virus on the 147th to 149th day of their adult life by allowing them to feed on a curly-top beet. After this, the insects were transferred to a healthy beet daily to the end of their natural life.

As is evident from table 8, leafhoppers may be reinfected with curly top during the latter part of their adult life, and apparently do not develop an immunity to the virus after once having been infective. The four insects reinfected by feeding them on a curly-top beet from the 147th to the 149th day of their adult life transmitted curly top to healthy

beets during the latter part of their natural life. As indicated in table 8, male No. 2 infected none of the 10 beets on which it fed during the 41st to 50th day, and also none of the 53 beets during the 94th to 146th day of its adult life. When, however, this male was reinfected by feeding on a curly-top beet for 3 days, it infected 4 of 37 beets on which it fed during the 150th to 186th day of its adult life. Male No. 3 infected only 1 of 10 beets on which it fed from the 41st to 50th day and only 2 of 53 beets during the 94th to 146th day of its adult life. After being reinfected by feeding for a period of 3 days on a curly-top beet, this leafhopper infected 5 of 36 beets on which it fed during the 150th to 185th day of its adult life.

In the second experiment, three infective insects which had completed their nymphal stages on a curly-top beet were confined on sweet corn for 105 days, and then each one was transferred to three successive healthy beets for periods of 14, 7, and 7 days, respectively. They were reinfected by allowing them to feed for 5 days on a curly-top beet from the 134th to 138th day of their adult life. The leafhoppers were then provided with a healthy beet daily for the remainder of their adult life.

The results in table 8 show that the three leafhoppers transmitted curly top to beets after being reinfected with the virus. Female No. 2 infected none of the three beets on each of which it fed for periods of 1 to 2 weeks during the 106th to 133rd day of its adult life. This insect was then reinfected by exposure to a curly-top beet, after which it infected 7 of 20 beets on which it fed during the 139th to 158th day of its adult life. Female No. 3, after 105 days on sweet corn, failed to infect a healthy beet on which it fed for 14 days, but infected 2 successive beets on each of which it was confined for a period of 7 days. This female was reinfected and subsequently infected 16 of 50 beets to which it was exposed during the 139th to 188th day of its adult life.

The results of other experiments would indicate that male No. 2 and females No. 1, No. 2, and No. 4 in table 8 were infective during the early part of their adult life although they produced no infections during the period of time that they were confined on beets. Preceding experiments on the transmission of curly top by insects that had completed their nymphal stages on a diseased beet (tables 4 and 5) show that the 28 leafhoppers were all infective. Figure 5 shows that in one experiment 5 of 12 of these infective insects produced no infection between the 41st and 50th day of their adult life, but all produced curly-top infections during a later period.

The seven leafhoppers in the present experiments probably had a greatly reduced infective capacity before they were reinfected on the

TABLE 8

## REINFECTION OF LEAFHOPPERS FED DURING NYMPHAL STAGES ON DISEASED BEETS WITH CURLY TOP DURING LATER ADULT LIFE

Insect sex and No.	Dates on host plant	Host plant	Number of days on host plant	Period of adult life on host plant, days	Adult age when infection produced, days
Experiment No. 1					
♂ 1	Apr. 9-May 19	Corn.....	40	1-40	.....
	May 19-May 29	Healthy beet.....	10*	41-50	41, 42, 43
	May 29-July 11	Corn.....	43	51-93	.....
	July 11-Sept. 2	Healthy beet.....	53*	94-146	121, 146
	Sept. 2-Sept. 5	Curly-top beet.....	3	147-149	.....
	Sept. 5-Sept. 20	Healthy beet.....	15*	150-164	157
♂ 2	Apr. 9-May 19	Corn.....	40	1-40	.....
	May 19-May 29	Healthy beet.....	10*	41-50	.....
	May 29-July 11	Corn.....	43	51-93	.....
	July 11-Sept. 2	Healthy beet.....	53*	94-146	.....
	Sept. 2-Sept. 5	Curly-top beet.....	3	147-149	.....
	Sept. 5-Oct. 12	Healthy beet.....	37*	150-186	156, 161, 165, 185
♂ 3	Apr. 9-May 19	Corn.....	40	1-40	.....
	May 19-May 29	Healthy beet.....	10*	41-50	42
	May 29-July 11	Corn.....	43	51-93	.....
	July 11-Sept. 2	Healthy beet.....	53*	94-146	103, 115
	Sept. 2-Sept. 5	Curly-top beet.....	3	147-149	.....
	Sept. 5-Oct. 11	Healthy beet.....	36*	150-185	155, 159, 172, 174, 179
♀ 1	Apr. 9-May 19	Corn.....	40	1-40	.....
	May 19-May 29	Healthy beet.....	10*	41-50	.....
	May 29-July 11	Corn.....	43	51-93	.....
	July 11-Sept. 2	Healthy beet.....	53*	94-146	.....
	Sept. 2-Sept. 5	Curly-top beet.....	3	147-149	.....
	Sept. 5-Oct. 11	Healthy beet.....	36*	150-185	154, 184
Experiment No. 2					
♀ 2	July 25-Nov. 7	Corn.....	105	1-105	.....
	Nov. 7-Nov. 21	Healthy beet.....	14	106-119	.....
	Nov. 21-Nov. 28	Healthy beet.....	7	120-126	.....
	Nov. 28-Dec. 6	Healthy beet.....	7	127-133	.....
	Dec. 6-Dec. 11	Curly-top beet.....	5	134-138	.....
	Dec. 11-Dec. 31	Healthy beet.....	20*	139-158	140, 141, 142, 151, 153, 154, 157
♀ 3	July 25-Nov. 7	Corn.....	105	1-105	.....
	Nov. 7-Nov. 21	Healthy beet.....	14	106-119	.....
	Nov. 21-Nov. 28	Healthy beet.....	7	120-126	120-126
	Nov. 28-Dec. 6	Healthy beet.....	7	127-133	127-133
	Dec. 6-Dec. 11	Curly-top beet.....	5	134-138	.....
	Dec. 11-Jan. 30	Healthy beet.....	50*	139-188	140, 145, 147, 148, 150, 152, 154, 159, 161, 164, 166, 169, 173, 175, 178, 187
♀ 4	July 25-Nov. 7	Corn.....	105	1-105	.....
	Nov. 7-Nov. 21	Healthy beet.....	14	106-119	.....
	Nov. 21-Nov. 28	Healthy beet.....	7	120-126	.....
	Nov. 28-Dec. 6	Healthy beet.....	7	127-133	.....
	Dec. 6-Dec. 11	Curly-top beet.....	5	134-138	.....
	Dec. 11-Jan. 10	Healthy beet.....	30*	139-168	154

\* Leafhopper transferred to healthy beet daily for number of days indicated.

134th and 147th day of their adult life. As shown in figure 5, the six leafhoppers still alive after the 130th day produced only one infection, although each insect was exposed to from 14 to 76 beets, or an average of 48 beets. The results presented in table 8 show that the 7 insects in the two experiments which were reinfected, produced from 1 to 16 infections, or an average of 4.6 infections, while feeding on from 15 to 50 beets, or an average of 32 beets, during their later adult life. The evidence clearly indicates that the beet leafhopper can be reinfected with the curly-top virus.

### INCUBATION PERIOD OF DISEASE IN SUGAR BEETS

Leafhoppers with a greater infective capacity might inoculate more virus into the plant during their feeding and for that reason there might be a shorter incubation period of the disease in the beets. In order to test this hypothesis a comparison was made of the incubation period of the disease infected by adults fed for periods of from 10 minutes to 3 hours on curly-top beets, with those infected by adults which had completed their nymphal stages on a source of virus.

The results, summarized in table 9, show that the incubation period of the disease in the first beet infected by previously noninfective adults which had fed for periods of from 10 minutes to 3 hours on a diseased beet varied from 6 to 35 days. The incubation period of the disease in the first beet infected by leafhoppers fed during all of their nymphal stages on diseased beets varied from 6 to 35 days (table 10). These results indicate no apparent differences in the length of the incubation period of the disease in the first beet infected by adults fed for varying periods on a diseased plant.

The results of table 9 indicate that the incubation period of the disease in successive infections by leafhoppers which had fed for only short periods during their adult life on a diseased beet varied from 5 to 39 days, while this period varied from 5 to 38 days with adults which completed the nymphal stages on diseased beets (table 10). The fluctuations were irregular, and the occasional prolonged periods are difficult to explain. The variation may be attributable to slight differences in size or resistance of the beets or in growing conditions.

It seems possible that, as the virus becomes more dilute in the insect with successive feedings on healthy plants, the incubation period of the disease in the beets would be increased. The minute quantity of virus injected into the feeding punctures by such insects as they slowly exhausted their store of virus would take a long time to multiply and cause symptoms of the disease to develop. The evidence obtained, however,

TABLE 9

INCUBATION PERIOD OF THE DISEASE IN BEETS INFECTED BY BEET LEAFHOPPERS  
FED SHORT PERIODS ON CURLY-TOP BEETS AND THEN PROVIDED  
WITH A HEALTHY BEET DAILY DURING ADULT LIFE

Time on curly-top beets, minutes	Insect sex and No.	Adult age when infection produced, days	Incuba- tion period of disease in beets, days	Time on curly-top beets, minutes	Insect sex and No.	Adult age when infection produced, days	Incuba- tion period of disease in beets, days
10	♀ 1	36	11	60	♀ 9	10	16
	♀ 3	15	27			12	15
		50	19			18	18
	♀ 4	9	35		♀ 10	7	10
		17	5			12	11
		18	13			18	37
		32	15		♀ 11	4	20
		1	9			21	*
	♂ 1	3	8			7	7
		9	8			17	18
		31	8			25	13
	♂ 2	5	12		♀ 12	102	*
20		48	39			107	9
		81	34			123	17
	♀ 5	7	14			130	21
		43	7			8	13
		45	7		♂ 8	10	5
	♀ 6	1	17			16	15
	♂ 3	15	6			23	11
		1	14		♂ 8	68	16
		9	11			9	35
	♂ 4	13	11		♀ 13	11	6
		14	12			35	8
		25	7			42	23
40		71	26			68	14
	♂ 5	7	11		♂ 9	77	9
		117	17			3	12
	♀ 7	7	11			9	14
		10	12		♂ 10	11	10
	♀ 8	115	10			16	15
		135	*			25	13
		161	*				
	♂ 6	6	32		♂ 10	1	11
		16	7			9	9
		18	7			13	8
		29	32			27	*
	♂ 7	34	8		♂ 11	29	17
		8	9			36	8
		17	19		♂ 12	42	13
						52	12
						67	*
						105	*

\* No records kept.

TABLE 10  
INCUBATION PERIOD OF DISEASE IN BEETS INFECTED BY BEET LEAFHOPPERS FED  
DURING NYMPHAL STAGES ON CURLY-TOP BEETS AND THEN PROVIDED  
WITH A HEALTHY BEET DAILY DURING ADULT LIFE

Insect sex and No.	Adult age when infection produced, days	Incubation period of disease in beets, days	Insect sex and No.	Adult age when infection produced, days	Incubation period of disease in beets, days
♀ 1	2	35	♀ 5	3	0
	6	18		5	15
	7	6		6	25
	8	35		7	*
	9	21		20	17
	13	14		25	7
	16	8		33	7
	29	16		48	8
	38	13		49	9
	50	38		64	25
♀ 2	6	10	♀ 6	73	31
	8	16		87	19
	9	11		1	24
	10	11		2	7
	13	6		3	13
	18	7		4	7
	20	15		5	12
	22	7		8	6
	26	17		9	11
	28	10		10	14
	29	23		13	11
	30	10		15	6
	31	14		19	8
	33	14		20	13
	34	15		22	9
	38	9		27	11
♀ 3	42	9		28	9
	44	11		29	8
	45	8		31	*
	46	17		35	12
	50	14		36	15
	83	17		56	39
	5	14		65	19
	6	7		67	9
	7	8		2	16
	9	5		5	21
♀ 4	16	12	♀ 7	6	10
	25	12		7	11
	26	7		9	16
	30	19		10	9
	40	11		12	12
	55	13		14	9
	59	25		15	7
	2	17		16	16
	7	10		19	8
	33	8		22	6
	80	32		24	6
				26	7
				30	*
				35	21
				48	18
				50	9

\* No records kept.

Table 10 concluded on page 338.

TABLE 10—(Concluded)

Insect sex and No.	Adult age when infection produced, days	Incubation period of disease in beets, days	Insect sex and No.	Adult age when infection produced, days	Incubation period of disease in beets, days
♀ 8	1	15	♂ 1	1	7
	2	27		2	9
	8	8		3	7
	15	8		6	21
	22	5		8	13
	23	5		9	7
	27	17		11	8
	30	9		12	11
	37	6		13	8
	60	*		16	10
	63	7		21	10
	64	10		23	14
	76	11		24	12
	77	5		26	10
	80	8		27	10
	82	7		30	13
	108	17		32	15
♀ 9	4	8	♂ 2	33	7
	5	7		39	13
	21	*		43	13
	29	8		44	10
	31	13		48	6
	42	29		49	15
	43	15		51	9
	45	15		58	9
	49	11		59	7
	50	13		2	6
	53	22		4	13
	54	9		5	12
	57	10		7	18
	100	26		9	12
	104	23		10	11
	1	20		11	9
	2	13		12	13
♀ 10	3	6		13	18
	4	9		14	8
	6	10		16	8
	22	10		17	7
	68	31		24	14
	151	13		27	11
				29	7
				31	7
				33	7

\* No records kept.

does not indicate a lengthening of the incubation period of the disease as the infective capacity of the insect is progressively decreased: the period in the last beet infected was usually no longer than for infections produced during the early adult life of the leafhopper.

### SUMMARY

An investigation was undertaken to determine whether the curly-top virus of the sugar beet multiplied in the beet leafhopper. If a multiplication occurs in the leafhopper, the insects probably should not only retain the infective capacity during their entire adult life, but those fed for only a short period on a diseased beet should also be able to cause as many infections as those fed for long periods. Accordingly, experiments were conducted in order to determine whether the infectivity of the insects remained constant during their adult life and whether the length of the feeding period on a diseased beet modified the infective capacity of the insects. Leafhoppers were fed for varying periods on curly-top beets and tested for their infectivity by transferring them daily to healthy beets. The daily transfers were considered frequent enough to prevent the insect from reinfecting itself with virus after infecting the healthy beet.

The results of the investigation indicate that the curly-top virus does not multiply in the beet leafhopper. No evidence was found to support such a theory as has often been surmised on the basis of indirect evidence.

There was a gradual decrease in the percentage of beets infected by the leafhopper during successive 30-day periods of adult life when the insects were transferred daily to a healthy beet. When adults or nymphs were fed for longer periods on diseased beets, they remained highly infective for a longer period than those which had fed short periods. The distribution of the infections caused by the beet leafhopper during its adult life failed to indicate a multiplication of the virus in the insect.

Many of the infective leafhoppers apparently lost the capacity to produce infection during late adult life; others retained their infectivity but infected beets only at great intervals. Insects which had completed the nymphal stages on curly-top beets were confined as adults on curly-top-immune sweet corn and were tested every 10th day of adult life for their capacity to transmit curly top. These insects showed a gradual loss from 50.0 per cent transmission on the 1st day to 3.1 per cent on the 90th day. If permanence of infective power during the life of the insect is the basis for the assumption that a multiplication of the virus occurs in the vector, then it cannot be argued that a multiplication of the curly-top virus occurs in the beet leafhopper.

A rather strong point against the theory that a multiplication of the curly-top virus occurs in the leafhopper was found in the comparative study of the number of infections produced by insects after short and long feeding periods on diseased beets. Insects which had fed only a short period of time on a curly-top beet were capable of producing only an average of 3.4 infections when transferred daily to successive healthy beets during adult life, while insects fed for long periods caused an average of 15.6 infections. Leafhoppers which had fed for short periods on curly-top beets probably accumulated less virus and consequently produced fewer infections than those which fed for long periods; these facts indicate that the leafhoppers are merely internal mechanical carriers.

Experiments were conducted to test the possibility that the infrequent transmissions during late adult life of the insect may have been due to the inability of old insects to transmit the disease readily. These experiments showed that leafhoppers first infected during the latter part of their adult life do not transmit the disease as frequently as those infected early in their lives. However, this decrease in infectivity is less than that which occurs in insects infected during early adult life and kept on healthy plants until the latter part of their lives. Some leafhoppers reinfected with the virus by feeding on a curly-top beet during later adult life transmitted the virus as readily as recently molted adults. The evidence shows that the beet leafhopper does not lose the ability to acquire or transmit the virus as it approaches old age and that the insect can be reinfected with the virus during late adult life.

While carrying out the experiments to determine the infective capacity of insects fed for short periods on diseased beets, great variation was found in the length of the period of delay in the development of the infective capacity. The "incubation period" or the period of time that elapsed between the initial feeding of from 10 minutes to 3 hours on a curly-top beet and the first infection by the leafhopper varied from 1 to 44 days, or an average of 9.6 days. The insects produced infections at very irregular intervals after the first infection. This result does not suggest a multiplication, since one would expect to get infections at regular intervals if there was an increase of virus in the insect.

A comparison of the transmission of curly top by male and female leafhoppers revealed no differences that could be considered significant in view of the great variation within each sex.

There was no significant difference in the length of the incubation period of the disease in beets infected by leafhoppers fed for varying periods on curly-top beets.

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## THE HERBICIDAL PROPERTIES OF BORON COMPOUNDS<sup>1</sup>

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### INTRODUCTION

IN THE FIELD of chemical weed control there is a constant demand for a reagent that will render the soil permanently sterile, for use on graveled driveways, parking areas, railroad right of ways, and similar areas where any plant growth is a nuisance. Although arsenic has proved most effective (14)<sup>4</sup> for this purpose, its use is always attended by a poison hazard. For this reason it seems desirable to find a soil sterilant that is nonpoisonous to man and animals. The known toxicity of boron compounds to plants suggests the possibility of their use for this purpose.

While it is recognized that toxic concentrations of boron occur in soils in certain regions in California and Nevada (19, 23, 32) and that the leaching of additional boron compounds into the underground waters in these regions is undesirable, there are large areas in these states, and others, where such a condition does not exist. In fact, as the data presented in this paper will show, one of the most promising uses for boron compounds is in the control of range weeds in the north-coast counties of California where the underground waters are not utilized for irrigation. It seems therefore that such materials may find extensive use in many places.

On the other hand, it is well to point out at the outset that wherever boron is present in toxic quantities in soils, and wherever crop plants may be affected, boron compounds should not be used in weed control. One of the principal objects of the work to be presented here was to determine the behavior of these substances in soils so that they might be

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handled with safety. From the results of the leaching studies, it is obvious that they would be useless, and possibly harmful, if applied below the water line in irrigation ditches and that they must be handled with caution on walks and drives beneath which the roots of trees and shrubs are growing. They should never be used in regions devoted to growing citrus and walnuts or even on range areas where the run-off contributes to the water supply of these regions. With these definite limitations in mind the studies that have been made are presented.

#### REVIEW OF LITERATURE

Although the toxic nature of boron compounds has been known since 1876 (6), relatively few attempts have been made to utilize them as herbicides. Thompson and Robbins (37), who tried borax and boric acid in their experiments on barberry eradication, did not consider them satisfactory. Other tests have been made (12, 26), but in no instance have boron compounds been recommended for use in weed control.

The literature on the toxicity of boron as a contaminating material in commercial fertilizers gives a different view. During the World War imports of potash from Germany were cut off, and a new supply had to be found. Among the sources were certain dry lakes in the desert regions of California. Salts extracted from these lake beds contained, besides potash and other elements, rather large percentages of borax. No great effort was made in the beginning to separate the borax completely from the potash; the possible effects of borax on plants were not fully realized.

As a consequence, serious crop injury resulted in certain of the eastern states, where large amounts of fertilizer are applied in the drill with the seed of such crops as cotton, corn, and potatoes. The injury having been traced to the borax present in the fertilizer, numerous investigations of the effect of borax on plants were conducted by state experiment stations and by the United States Department of Agriculture. The reports of these investigations give much valuable information on the toxic effects of boron compounds. This work will be summarized as it relates to the use of boron in weed control.

**Boron as a Plant Poison.**—Judging from tests on the relative toxicity of different compounds of boron (6, 8, 38), it is the borate ion that produces the injury; the various compounds, therefore, are toxic in proportion to the elemental boron they carry and are comparable on that basis. Even relatively insoluble compounds of boron are toxic (20) if available to the plants. From this we may conclude that the toxicity of these compounds is not related to any peculiarities of chemical combination or molecular structure as is the case with chlorates and thiocyanates; con-

sequently, boron compounds would not be subject to chemical decomposition that might tend to reduce their toxicity within the soil. Since the presence of boron in the soil solution in toxic quantities should render a soil sterile, the solubility of the compounds present will be the principal factor governing their effectiveness.

The highly toxic nature of boron is shown by many tests. Solution-culture experiments (5, 6, 8, 20, 22, 27, 36, 38) indicate that the lower limit of boron toxicity ranges from 1 to 10 parts per million, according to the plant species; and all cultures containing 1,000 p.p.m. or above were lethal.

Sand cultures (6, 8) showed critical concentrations of the same order but even lower lethal concentrations. Pot-culture tests with soils (2, 3, 6, 8, 9, 11, 12, 20, 24, 38) vary because of the different soil types used and the different methods of application. The critical concentrations, where they could be calculated, were in the neighborhood of 10 to 15 p.p.m. on the basis of the air-dry soil. Fifty p.p.m. definitely affected germination, and 100 p.p.m. prevented germination entirely.

Field trials reported (6, 7, 10, 12, 29, 33, 35, 36, 38) indicate wide differences in the susceptibility of plant species and also variations due to soil type, precipitation, and method of application. In most cases dosages of 200 pounds per acre or more of borax were lethal, and injury resulted when as little as 5 to 20 pounds was applied in the drill with the seed. In terms of elemental boron, 15 p.p.m. in the soil produced injury, and 400 p.p.m. was lethal.

The problem of the susceptibility of different cultivated plants to boron injury has received considerable attention (4, 6, 7, 19, 20, 23, 25, 31, 32). Scofield and Wilcox (32) group a number of crop plants according to their boron tolerance, using walnuts and citrus as standards. Eaton (19) lists 55 plants in the order of their boron tolerance. Obviously, wide differences exist in the ability of different species to tolerate boron in the soil. This factor must be considered in the use of any material for weed control; an application that fails to sterilize the soil completely is certain to cause a shift in the flora, the more tolerant species surviving. If these species are less desirable than the original mixed flora, then little or nothing is accomplished by the treatment.

Studies on the effects of the method of applying boron compounds show that the severest injury results from a concentration of the poison in the surface soil where the young absorbing roots come into direct contact with it (34, 35). A delay between fertilizer application and seeding, heavy rains immediately after application, or a thorough mixing of the chemical with a large mass of soil all tended to reduce boron toxicity.

These observations indicate that soluble boron compounds are leached from the soil and that they are rapidly rendered unavailable to plants upon contact with a large volume of soil. The effect of soil type upon availability of boron is also important in relation to the amount required to sterilize different soils. Previous studies on this problem (9, 10, 18, 19, 27, 28, 32) indicate that toxicity is greatest in coarse-textured soils. The results to be presented substantiate this conclusion and indicate the magnitude of the differences to be found.

As a number of workers (3, 6, 7, 12, 13, 20, 35) have observed, the toxic effects of an application of boron to the soil diminish appreciably with time. It seems evident not only that soluble boron compounds leach from the soil, as reported by Kelley and Brown (23), but that those remaining are gradually rendered unavailable to plants (6). Both these properties tend to reduce the effectiveness of boron as a soil sterilant, and they definitely limit the time that a given application will last.

The foregoing consideration of the work on boron toxicity shows why this material has not been recommended in weed control. Although very toxic to certain plants, it is much less harmful to others; it loses in potency soon after application, both by leaching and by fixation. Furthermore, it has not been promoted by any commercial agency and consequently has not been tried under a wide range of conditions.

With the proper criteria for judging the herbicidal value of a reagent and the necessary studies on its properties, it should be possible to find a place for so toxic a chemical as boron in a comprehensive program of weed control. Studies made within recent years on herbicides (1, 14, 15, 16, 17, 21) go far toward establishing the standards to be met by a weed killer. From their chemical nature it is evident that compounds of boron would find legitimate use only in the practice of soil sterilization where an agent nonpoisonous to man and animals is required. The studies reported herein show how closely these compounds meet the standards for such an agent.

#### GREENHOUSE STUDIES ON THE TOXICITY AND FIXING OF BORAX IN FOUR CALIFORNIA SOILS

*Toxicity Studies.*—Studies on the toxicity and fixing of borax were begun in the summer of 1933 and have continued for over two years. The present paper reports the results of a number of these. This work aimed to discover a possible use for boron compounds in the process of chemical soil sterilization and was therefore established on a basis almost diametrically opposed to that of others who have studied boron injury. Since it was hoped to make immediate and practical use of the informa-

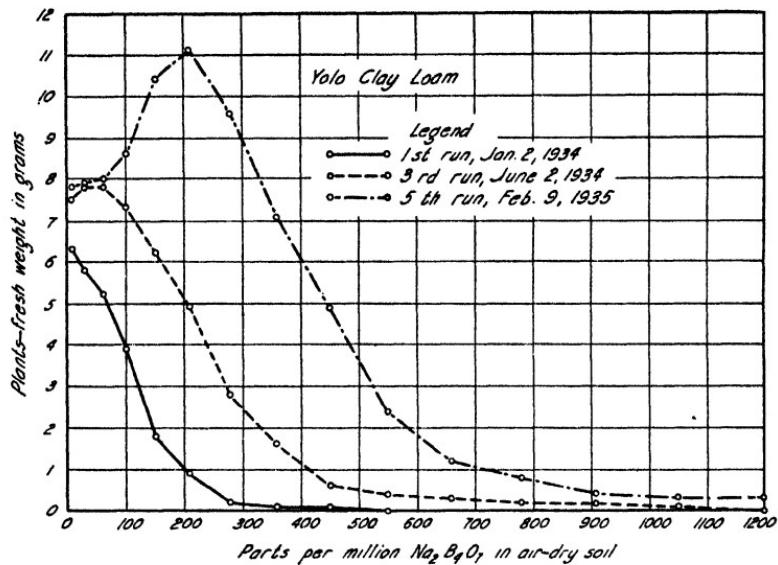


Fig. 1.—The relation of crop yield to the concentration of anhydrous borax in Yolo clay loam.

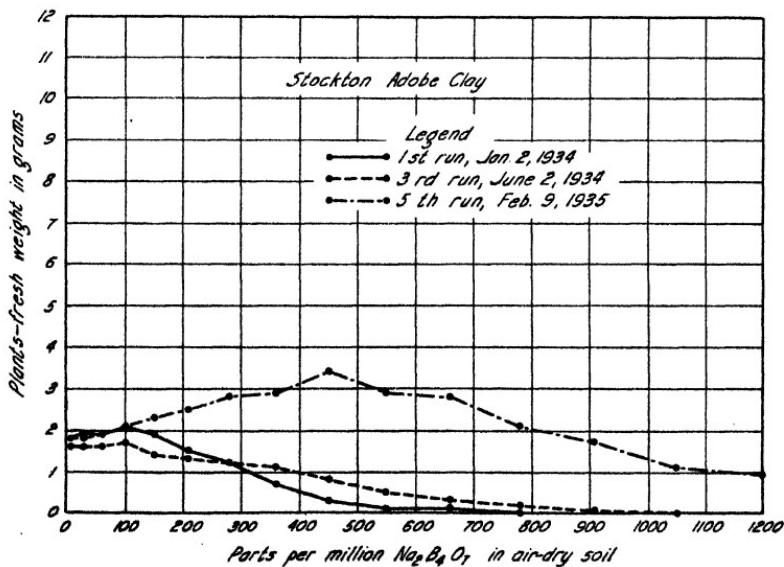


Fig. 2.—The relation of crop yield to the concentration of anhydrous borax in Stockton adobe clay.

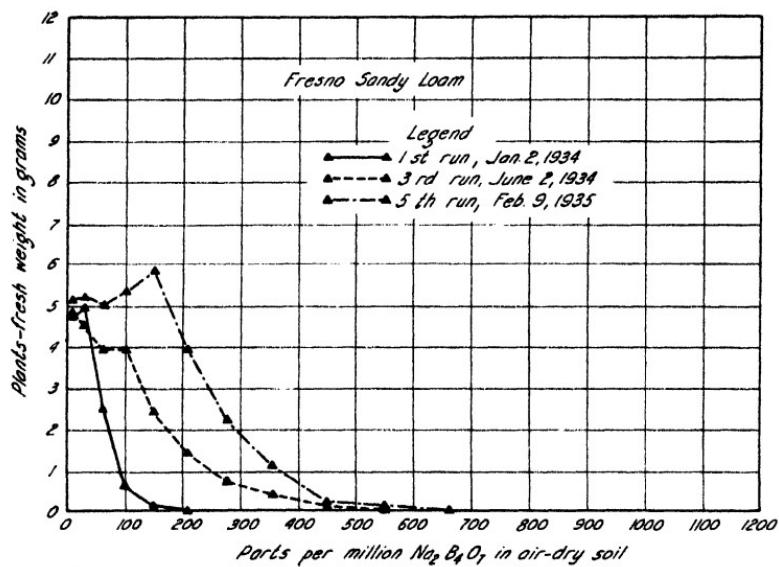


Fig. 3.—The relation of crop yield to the concentration of anhydrous borax in Fresno sandy loam.

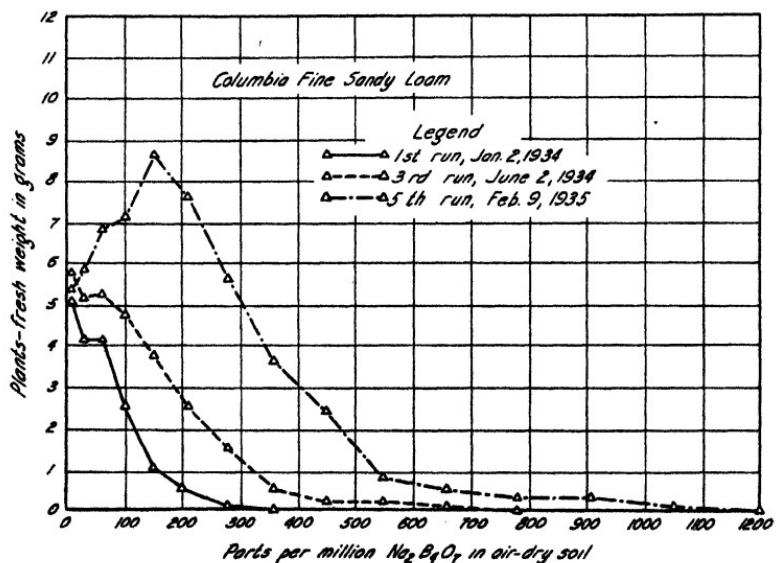


Fig. 4.—The relation of crop yield to the concentration of anhydrous borax in Columbia fine sandy loam.

TABLE 1

## TOXICITY OF SODIUM BORATE IN FOUR CALIFORNIA SOILS, AS SHOWN BY GROWTH OF INDICATOR PLANTS

Sodium borate expressed as p.p.m. anhydrous borax in the air-dry soil	Yolo clay loam		Stockton adobe clay		Fresno sandy loam		Columbia fine sandy loam	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.

First run, harvested January 2, 1934

	cm	gm	cm	gm	cm	gm	cm	gm
10.....	34	6.3	20	1.8	31	4.7	31	5.0
30.....	34	5.8	20	1.9	30	4.9	30	4.1
60.....	32	5.2	21	1.9	20	2.5	29	4.1
100.....	28	3.9	22	2.1	7	0.6	21	2.5
150.....	16	1.8	20	1.9	2	0.1	12	1.0
210.....	9	0.9	13	1.5	0	0.0	6	0.5
280.....	3	0.2	11	1.2	0	0.0	1	0.1
360.....	1	0.1	7	0.7	0	0.0	0	0.0
450.....	1	0.1	4	0.3	0	0.0	0	0.0
550.....	0	0.0	2	0.1	0	0.0	0	0.0
660.....	0	0.0	1	0.1	0	0.0	0	0.0
780.....	0	0.0	0	0.0	0	0.0	0	0.0
910.....	0	0.0	0	0.0	0	0.0	0	0.0
1050.....	0	0.0	0	0.0	0	0.0	0	0.0
1200.....	0	0.0	0	0.0	0	0.0	0	0.0
1360.....	0	0.0	0	0.0	0	0.0	0	0.0
Check.....	35	6.6	19	1.8	31	5.2	31	5.0
Check.....	34	6.3	20	2.0	31	5.2	31	4.9
Check.....	33	6.4	18	1.6	28	4.4	29	4.9
Check.....	32	6.3	19	1.7	31	5.2	28	4.7

Third run, harvested June 2, 1934

	cm	gm	cm	gm	cm	gm	cm	gm
10.....	35	9.7	21	2.9	26	4.3	31	7.0
30.....	37	10.1	22	3.0	28	4.1	32	6.3
60.....	30	10.1	22	3.0	27	3.5	33	6.4
100.....	39	9.5	23	3.1	26	3.5	34	5.8
150.....	36	8.0	22	2.5	24	2.2	31	4.5
210.....	33	6.4	22	2.3	16	1.2	27	3.1
280.....	27	3.6	23	2.2	13	0.6	21	1.8
360.....	24	2.1	22	2.0	10	0.4	15	0.6
450.....	15	0.8	19	1.4	7	0.1	10	0.3
550.....	14	0.5	17	0.8	0	0.0	8	0.2
660.....	12	0.3	11	0.5	0	0.0	7	0.1
780.....	10	0.2	10	0.3	0	0.0	0	0.0
910.....	10	0.2	8	0.2	0	0.0	0	0.0
1050.....	6	0.1	6	0.1	0	0.0	0	0.0
1200.....	0	0.0	0	0.0	0	0.0	0	0.0
1360.....	0	0.0	0	0.0	0	0.0	0	0.0
Check.....	33	8.2	23	3.4	25	4.3	29	6.2
Check.....	35	9.9	23	3.3	27	4.8	28	6.8
Check.....	29	7.7	20	3.2	25	4.0	29	5.7
Check.....	29	7.4	21	3.2	29	4.9	29	5.3

TABLE 1—*Concluded*

Sodium borate expressed as p.p.m. anhydrous borax in the air-dry soil	Yolo clay loam		Stockton adobe clay		Fresno sandy loam		Columbia fine sandy loam	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
Fifth run, harvested February 9, 1935								
	cm	gm	cm	gm	cm	gm	cm	gm
10.....	28	4.6	15	1.6	22	2.8	23	3.6
30.....	29	4.7	15	1.6	23	2.8	25	3.9
60.....	29	4.7	15	1.6	23	2.7	28	4.5
100.....	31	5.1	16	1.8	24	2.9	30	5.0
150.....	33	6.2	17	2.0	27	3.1	34	5.7
210.....	35	6.6	19	2.2	24	2.1	34	5.1
280.....	34	5.7	21	2.5	17	1.2	31	3.7
360.....	32	4.2	23	2.5	13	0.6	25	2.4
450.....	28	2.9	24	3.0	7	0.2	22	1.6
550.....	20	1.4	22	2.5	5	0.1	13	0.5
660.....	13	0.7	22	2.4	0	0.0	10	0.5
780.....	11	0.5	18	1.9	0	0.0	7	0.2
910.....	8	0.2	16	1.5	0	0.0	6	0.2
1050.....	7	0.2	13	0.9	0	0.0	5	0.1
1200.....	7	0.2	12	0.8	0	0.0	0	0.0
1360.....	5	0.1	10	0.4	0	0.0	0	0.0
Check.....	26	4.0	15	1.7	23	2.7	24	3.7
Check.....	26	4.0	14	1.7	22	2.9	23	3.6
Check.....	23	3.4	14	1.4	21	2.6	21	2.8
Check.....	25	3.5	14	1.5	21	2.5	21	2.9

tion gained, a biological testing method was used which measured the toxicity of the applied chemical directly without resort to chemical analysis and subsequent interpretation. The results, therefore, are not so strictly quantitative as might be desired by some; but they have nevertheless provided valuable information and have aided greatly in the interpretation of field-plot data.

The method used has been described in a paper on the toxicity of sodium arsenite and sodium chlorate in California soils (17). Briefly, it consists of series of pot cultures grown in the greenhouse set up in No. 2 cans and containing increasing amounts of borax within each series. The soils used were Yolo clay loam, Stockton adobe clay, Fresno sandy loam, and Columbia fine sandy loam. Each culture in the Fresno sandy loam contained 600 grams of soil, and each in the other three soils contained 500 grams.

In making up the cultures the necessary amount of borax was applied, dissolved in a volume of water sufficient to bring the soil to field capacity. The actual moistening was done in three rapid stages so that the moisture was distributed within one minute or less. Previous tests with arsenic and chlorate had shown this to be a satisfactory method, giving a uniform distribution of the chemical.

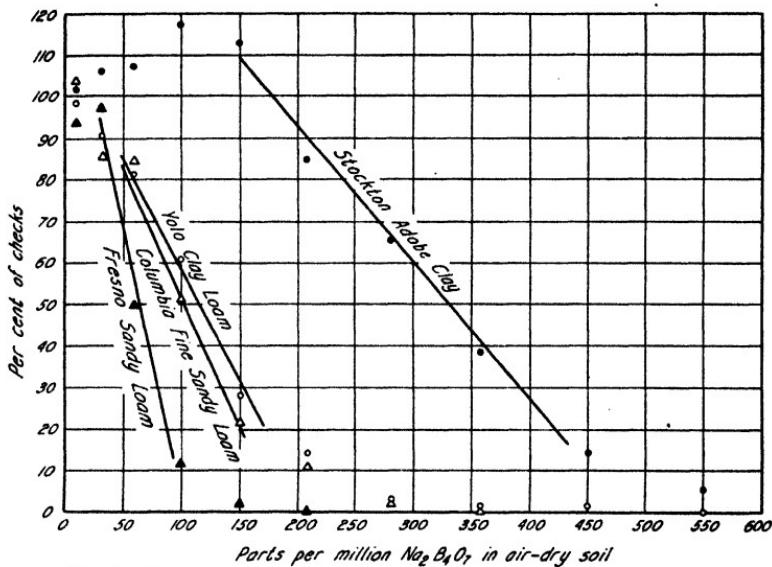


Fig. 5.—The relation of crop yield, expressed as per cent of the checks, to the concentration of anhydrous borax in four soils.

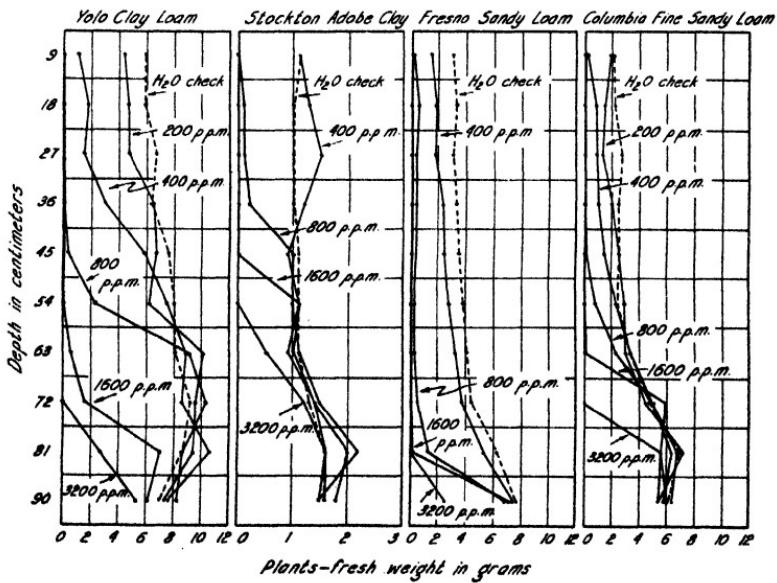


Fig. 6.—The relation of crop yield to penetration of borax into columns of four California soils, showing their power to retain this chemical.

The moistened soil was planted with Kanota oats, 13 seeds to a can. After about ten days the seedlings were thinned to 10 in each can. Thirty days from planting they were harvested, the fresh weight of tops being taken as a measure of the effect of the chemical upon growth. After har-

TABLE 2  
THE FIXING POWER OF YOLO CLAY LOAM FOR SODIUM BORATE,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS  
(Harvested December 15, 1934)\*

Fraction of soil column	H <sub>2</sub> O check		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 100 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 200 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 400 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0- 9.....	24	5.9	28	5.2	24	4.3	11	1.0	0	0.0
9-18.....	24	5.9	20	5.4	27	4.7	17	1.8	0	0.0
18-27.....	24	6.7	27	5.9	26	4.7	17	1.4	0	0.0
27-36.....	24	6.3	24	5.0†	29	6.5	20	2.9	0	0.0
36-45.....	25	7.6	25	6.6	27	6.7†	28	5.9†	5	0.3
45-54.....	26	8.0	24	6.7	26	6.2	29	7.5	21	2.2
54-63.....	27	8.1	30	9.7	29	10.1	28	8.9	32	9.1†
63-72.....	27	9.2	28	11.4	27	9.6	28	8.6	27	10.4
72-81.....	26	8.7	28	11.2	28	9.5	30	10.7	27	8.7
81-90.....	24	7.0	28	7.8	24	7.4	25	7.7	27	8.2

Fraction of soil column	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 1,600 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 3,200 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 100 p.p.m.†		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 400 p.p.m.†		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 1,600 p.p.m.‡	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0- 9.....	0	0.0	0	0.0	26	4.0	8	0.5	0	0.0
9-18.....	0	0.0	0	0.0	26	4.5	19	1.7	0	0.0
18-27.....	0	0.0	0	0.0	28	4.6	14	1.3	0	0.0
27-36.....	0	0.0	0	0.0	25	4.1†	19	2.1	0	0.0
36-45.....	0	0.0	0	0.0	27	4.6	24	3.8	0	0.0
45-54.....	0	0.0	0	0.0	21	3.2	24	4.0†	0	0.0
54-63.....	5	0.5	0	0.0	19	4.1	17	2.2	0	0.0
63-72.....	17	1.6	0	0.0	19	2.3	17	2.3	12	0.8
72-81.....	26	7.0†	21	2.7	19	3.0	18	2.9	21	2.4
81-90.....	25	6.1	21	5.4†	20	3.3	20	3.1	23	3.8†

\* Average weight of plants in 10 untreated checks = 9.8 gm.

† Boron injury present to this depth.

‡ Soil in the last three columns moistened before the sodium borate was applied.

vest the tops were returned to the cans and were dried along with the soil for thirty days. The soils were then repulverized and returned to the cans, the dried tops being included under the soils. After moistening, the cultures were planted and carried along as in the first cropping. Five croppings are reported in this paper. The cultures are still on hand and will be cropped several more times. From the standpoint of weed control, the more important results are probably included in this report.

All cultures were replicated five times; and the points given in figures 1, 2, 3, and 4 on toxicity represent the average of the five. The figures mentioned present the data of the first, third, and fifth crops representative of the results on these soils; and table 1 gives the yields of these

TABLE 3  
THE FIXING POWER OF STOCKTON ADOBE CLAY FOR SODIUM BORATE,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS  
(Harvested January 14, 1935)\*

Fraction of soil column	H <sub>2</sub> O check		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 100 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 200 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 400 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-9.....	14	1.1	14	1.1	14	1.3	11	1.1	0	0.0
9-18.....	13	1.0	14	1.0	14	1.1	12	1.3	3	0.1
18-27.....	13	1.0	13	0.9†	13	1.0	14	1.5	3	0.1
27-36.....	12	1.0	13	1.0	13	0.8†	13	1.2	4	0.2
36-45.....	13	1.1	12	1.0	14	1.1	13	0.9†	11	1.0
45-54.....	14	1.1	15	1.1	14	1.1	14	1.1	14	1.0†
54-63.....	14	1.1	14	1.2	14	1.2	12	0.9	14	1.1
63-72.....	15	1.3	17	1.9	15	1.3	16	1.4	16	1.5
72-81.....	16	1.6	16	1.6	19	2.0	16	1.6	18	2.2
81-90.....	16	1.6	17	1.8	17	1.8	17	1.5	18	1.5

Fraction of soil column	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 1,600 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 3,200 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 100 p.p.m.‡		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 400 p.p.m.‡		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 1,600 p.p.m.‡	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-9.....	0	0.0	0	0.0	17	1.4	16	1.5	0	0.0
9-18.....	0	0.0	0	0.0	15	1.0	14	1.4	0	0.0
18-27.....	0	0.0	0	0.0	14	1.1†	13	1.4	0	0.0
27-36.....	0	0.0	0	0.0	14	1.1	16	1.4	0	0.0
36-45.....	0	0.0	0	0.0	13	1.0	16	0.9†	0	0.0
45-54.....	12	1.2	0	0.0	14	1.1	15	1.0	8	0.3
54-63.....	14	1.0†	7	0.5	14	1.0	13	0.9	13	1.3
63-72.....	16	1.4	15	1.2	14	1.0	14	0.9	15	1.0†
72-81.....	18	2.0	15	1.6†	14	1.2	12	0.8	14	1.0
81-90.....	17	1.8	17	1.6	15	1.2	14	1.1	13	0.9

\* Average weight of plants in 10 untreated checks = 1.5 gm.

† Boron injury present to this depth.

‡ Soil in the last three columns moistened before the sodium borate was applied.

crops. The relative toxicities in the four soils, and the loss of toxicity with time and cropping are shown. The curves for each soil have been reduced to a common base by multiplying values in the third and fifth runs by the average weight of first-run checks and dividing by the average weight of checks for the respective run.

In the coarser soils, evidently, borax is very toxic. The oat plant, being located at about the middle of the list of plants given by Eaton (19), is

intermediate in susceptibility to boron injury. The results of these tests show that boron, expressed as anhydrous borax, ranks in effectiveness with trivalent arsenic and sodium chlorate. The most pronounced difference occurs in Stockton adobe clay, where the toxicity of borax is notably low and the loss of toxicity with time and cropping very great. To study

TABLE 4  
THE FIXING POWER OF FRESNO SANDY LOAM FOR SODIUM BORATE,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS  
(Harvested February 20, 1935)\*

Fraction of soil column	H <sub>2</sub> O check		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 100 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 200 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 400 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0- 9.....	26	2.9	25	2.3	24	2.5	18	1.4	3	0.1
9-18.....	23	3.2	23	2.6	25	2.5	22	1.9	10	0.5
18-27.....	25	3.0	26	2.9	26	2.8	21	1.8	8	0.3
27-36.....	25	3.2	26	2.9	27	3.0	26	2.3	8	0.3
36-45.....	26	3.4	26	3.2†	28	3.1	24	2.4	8	0.3
45-54.....	28	3.8	27	3.2	27	3.2	25	2.7	5	0.2
54-63.....	27	4.1	28	3.4	29	3.7	27	3.2	5	0.2
63-72.....	28	4.3	29	4.0	29	3.9†	29	3.7	9	0.4
72-81.....	31	6.2	31	4.5	31	5.6	31	5.3†	17	1.3†
81-90.....	33	7.6	36	6.9	35	7.5	34	7.4	33	6.8
<hr/>										
Fraction of soil column	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 1,000 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 3,200 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 100 p.p.m.†		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 400 p.p.m.†		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 1,600 p.p.m.†	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0- 9.....	0	0.0	0	0.0	26	2.3	19	1.4	0	0.0
9-18.....	0	0.0	0	0.0	23	2.2	24	2.2	0	0.0
18-27.....	0	0.0	0	0.0	23	2.1	22	1.8	0	0.0
27-36.....	0	0.0	0	0.0	25	2.4	22	2.1	0	0.0
36-45.....	0	0.0	0	0.0	25	2.7†	23	2.3	0	0.0
45-54.....	0	0.0	0	0.0	27	2.9	26	2.5	0	0.0
54-63.....	0	0.0	0	0.0	28	3.4	23	2.3	0	0.0
63-72.....	0	0.0	0	0.0	27	3.7	27	2.7	0	0.0
72-81.....	8	0.1†	0	0.0	22	3.0	23	2.0†	9	0.3
81-90.....	30	7.2	27	2.5†	23	2.0	23	2.4	23	2.3†

\* Average weight of plants in 10 untreated checks = 4.8 gm.

† Boron injury present to this depth.

‡ Soil in the last three columns moistened before the sodium borate was applied.

the relation of toxicity and textural grade of the soil, the curves of the first runs have been reproduced, expressed on the basis of percentage of their checks. These curves appear in figure 5. Apparently a rough correlation exists between toxicity and particle size, as shown by the slopes of these curves. Considering the difference in the water-holding capacity of these soils, it seems that the differences in the three coarser

soils may be explained on the basis of differences in the concentration of borax in the soil solution. In the Stockton adobe clay, some other factor reduces the toxicity. This soil is highly colloidal but, under the conditions of this experiment, not particularly productive. Evidently it renders much of the applied borax unavailable to plants.

TABLE 5  
THE FIXING POWER OF COLUMBIA FINE SANDY LOAM FOR SODIUM BORATE,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS  
(Harvested February 4, 1935)\*

Fraction of soil column	H <sub>2</sub> O check		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 100 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 200 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 400 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0- 9.....	16	2.1	16	1.9	18	1.9	4	0.2	0	0.0
9-18.....	16	2.1	17	2.2	14	1.4	10	0.8	0	0.0
18-27.....	16	2.7	16	2.1	13	1.2	12	0.9	0	0.0
27-36.....	16	2.4	17	2.3†	17	2.0	13	0.9	0	0.0
36-45.....	16	2.5	17	2.4	20	2.4†	16	1.4	2	0.1
45-54.....	16	2.4	17	3.0	17	2.9	19	2.3†	10	0.7
54-63.....	19	3.3	17	3.0	17	2.9	19	3.3	19	2.3†
63-72.....	24	4.7	23	4.3	24	4.4	24	4.9	25	4.8
72-81.....	28	6.9	27	6.8	28	7.2	28	6.7	28	6.3
81-90.....	27	6.1	28	7.0	26	5.4	26	6.3	26	5.7
Fraction of soil column	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 1,600 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 3,200 p.p.m.		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 100 p.p.m.†		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 400 p.p.m.†		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 1,600 p.p.m.†	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0- 9.....	0	0.0	0	0.0	14	1.5	6	0.4	0	0.0
9-18.....	0	0.0	0	0.0	14	1.4	9	0.7	0	0.0
18-27.....	0	0.0	0	0.0	15	1.6†	12	0.9	0	0.0
27-36.....	0	0.0	0	0.0	14	1.8	15	1.6	0	0.0
36-45.....	0	0.0	0	0.0	14	1.5	16	1.8	0	0.0
45-54.....	0	0.0	0	0.0	13	1.4	15	1.4	0	0.0
54-63.....	2	0.1	0	0.0	15	1.6	15	1.5	0	0.0
63-72.....	27	5.9†	0	0.0	15	1.8	15	1.6†	6	0.2
72-81.....	25	5.5	26	5.5†	15	1.5	15	1.9	11	0.9
81-90.....	27	5.4	28	6.0	18	2.0	16	1.8	17	1.6†

\* Average weight of plants in 10 untreated checks = 6.5 gm.

† Boron injury present to this depth.

‡ Soil in the last three columns moistened before the sodium borate was applied.

Another interesting feature of these tests is the large loss of toxicity in the later runs. In every soil, by the fifth run, concentrations which had initially rendered the soil sterile have produced crops as good as the checks or better. In every soil also there is a noticeable stimulation of growth in the lower concentrations. After the harvest of the third run on June 2, 1934, the cultures stood in the greenhouse at Davis until Septem-

ber 18. During this time the loss of available borax was particularly noticeable.

*Soil-Tube Studies.*—To study the fixing or retention of borax in an available form by soils, tests were made using a special type of soil tube.

TABLE 6  
RESULTS OF LEACHING SODIUM BORATE IN YOLO CLAY LOAM,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS  
(Harvested March 23, 1935)\*

Fraction of soil column	Check		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.					
	10 cm H <sub>2</sub> O		0 cm H <sub>2</sub> O		2.5 cm H <sub>2</sub> O		5.0 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm
0-9	19	4.4	0	0.0	0	0.0	0	0.0
9-18	21	4.6	0	0.0	0	0.0	4	0.2
18-27	22	5.5	0	0.0	0	0.0	3	0.2
27-36	22	5.5	0	0.0	0	0.0	0	0.0
36-45	22	6.1	8	0.5	0	0.0	4	0.2
45-54	23	6.5	16	1.6	17	1.3	9	0.8
54-63	22	6.1	24	6.8†	23	4.8†	17	1.6
63-72	22	4.9	23	8.5	22	8.8	27	7.1†
72-81	22	4.7	21	8.7	29	10.1	26	9.3
81-90	19	4.3	22	8.0	25	9.5	24	7.3
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.								
Fraction of soil column	10 cm H <sub>2</sub> O		20 cm H <sub>2</sub> O		40 cm H <sub>2</sub> O		80 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
	cm	gm	cm	gm	cm	gm	cm	gm
0-9	22	3.1	22	3.6	20	3.3	21	3.4
9-18	15	1.4	20	3.5	21	4.3	21	4.4
18-27	6	0.4	20	3.4	22	4.1	22	3.4†
27-36	0	0.0	18	2.4	21	3.8	21	4.3
36-45	0	0.0	7	0.5	21	2.5	21	4.5
45-54	0	0.0	4	0.2	23	3.1	19	3.0
54-63	5	0.2	6	0.3	21	3.4	19	2.7
63-72	28	5.2	8	0.6	22	2.9	19	2.6
72-81	24	5.1†	18	1.8	11	0.7	20	2.7
81-90	22	5.0	18	2.2†	7	0.3†	20	2.9

\* Average weight of plants in 10 untreated checks = 8.2 gm.

† Boron injury present to this depth.

‡ Boron injury present below this depth.

This consists of a sheet of celluloid bent to form a hollow cylinder. It is supported on the outside with a sheet of ½-inch-mesh hardware cloth bent around it and wired in place. The tube, filled with air-dry soil, is moistened slowly by dripping a given solution on the soil through a small glass jet. The jets are adjusted to deliver 12 drops per minute, and the tubes are usually moistened in 24 to 36 hours, according to the field

capacity of the soil being studied. The solutions used in these tests contained 100, 200, 400, 800, 1,600, and 3,200 p.p.m. anhydrous borax, respectively; and the same four soils were used. A check tube moistened with water was included in each set.

TABLE 7  
RESULTS OF LEACHING SODIUM BORATE IN STOCKTON ADOBE CLAY,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS  
(Harvested April 22, 1935)\*

Fraction of soil column	Check		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.					
	10 cm H <sub>2</sub> O		0 cm H <sub>2</sub> O		2.5 cm H <sub>2</sub> O		5.0 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm
0- 0.....	16	1.7	9	0.2	10	0.5	20	1.3
0-18.....	16	2.0	10	0.3	8	0.4	10	0.7
18-27.....	16	1.7	12	0.5	8	0.4	6	0.3
27-36.....	16	1.8	19	1.2	7	0.6	8	0.4
36-45.....	18	2.2	18	1.1	18	1.0	12	0.8
45-54.....	19	2.1	19	1.5	19	1.2	18	1.2
54-63.....	18	2.2	16	1.4†	18	1.6	20	1.7
63-72.....	18	1.9	17	1.6	20	1.7†	17	1.9†
72-81.....	18	2.1	19	1.9	20	2.6	19	2.5
81-90.....	17	2.3	19	2.6	19	2.4	20	2.5
Fraction of soil column	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.							
	10 cm H <sub>2</sub> O		20 cm H <sub>2</sub> O		40 cm H <sub>2</sub> O		80 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm
0- 9.....	15	1.0	16	1.4	17	1.7	15	1.8
9-18.....	18	1.1	15	1.3	15	1.4	14	1.5†
18-27.....	11	0.5	16	1.2	16	1.5	13	1.4
27-36.....	12	0.7	16	1.1	17	1.4	14	1.2
36-45.....	12	0.7	16	1.2	18	1.3	16	1.5
45-54.....	14	0.9	18	1.4	17	1.3	16	1.2
54-63.....	19	1.3	19	1.5	19	1.2	19	1.4
63-72.....	19	1.6	17	1.2	19	1.2	20	1.3
72-81.....	19	2.3	19	1.5	17	1.2	19	1.2
81-90.....	17	2.1†	16	1.2†	16	1.0†	16	1.1

\* Average weight of plants in 10 untreated checks = 1.8 gm.

† Boron injury present to this depth.

‡ Boron injury present below this depth.

After moistening, the tubes were allowed to stand for 48 hours to approach equilibrium. Then, while lying in a horizontal position, each tube was opened, the soil column divided into 10 fractions of equal length, and each fraction mixed and placed in a No. 2 can. These cultures were seeded and handled as were those of the previous experiments. Ten cans

of air-dry soil were moistened and seeded with each set of soil tubes. The average yield of these untreated checks is given at the foot of each table. The results of these tests are presented in tables 2, 3, 4, and 5. The more characteristic data are shown graphically in figure 6.

TABLE 8

RESULTS OF LEACHING SODIUM BORATE IN FRESNO SANDY LOAM,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS  
(Harvested April 12, 1935)\*

Fraction of soil column	Check		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.					
	10 cm H <sub>2</sub> O		0 cm H <sub>2</sub> O		2.5 cm H <sub>2</sub> O		5.0 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm
0-9.....	21	3.1	9	0.2	20	1.1	20	1.8
9-18.....	18	2.7	10	0.2	15	0.6	18	1.2
18-27.....	18	2.7	0	0.0	6	0.1	14	0.7
27-36.....	20	3.3	5	0.1	6	0.5	7	0.6
36-45.....	20	3.1	4	0.1	7	0.4	6	0.4
45-54.....	21	3.6	4	0.2	4	0.2	4	0.2
54-63.....	21	3.4	3	0.1	4	0.2	11	0.7
63-72.....	22	3.8	9	0.5	6	0.3	7	0.6
72-81.....	21	4.2	23	2.0†	10	0.9	8	0.8
81-90.....	22	3.2	25	4.7	14	1.0†	10	0.6†
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.								
Fraction of soil column	10 cm H <sub>2</sub> O		20 cm H <sub>2</sub> O		40 cm H <sub>2</sub> O		80 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
	cm	gm	cm	gm	cm	gm	cm	gm
0-9.....	21	1.7	17	2.3†	19	2.7	18	2.4
9-18.....	22	2.5	22	2.8	19	2.6	18	2.5
18-27.....	21	2.0	21	2.4	19	2.4	19	2.6
27-36.....	22	1.5	20	2.3	18	2.5†	17	2.4
36-45.....	7	0.5	21	2.1	17	1.8	17	2.1
45-54.....	4	0.1	24	2.1	17	1.9	18	2.1
54-63.....	7	0.5	24	2.5	19	2.2	16	2.1
63-72.....	0	0.0	24	2.4	20	2.1	19	2.4
72-81.....	0	0.0	8	0.6	19	1.8	19	2.7
81-90.....	0	0.0†	10	0.5	22	2.4	23	2.0

\* Average weight of plants in 10 untreated checks = 5.4 gm.

† Boron injury present to this depth.

‡ Boron injury present below this depth.

Evidently, borax is held in the soil in an available form and tends to accumulate in the upper portion of the tube to which it is applied. Comparison with results of similar tests on sodium chlorate and sodium arsenite places borax between these other two chemicals in the firmness with which it is held.

As borax is somewhat less toxic than chlorate under the conditions of this experiment, the accumulation from the lower concentrations is less noticeable. In the higher concentrations, however, the results are more clear-cut. Probably the most outstanding difference occurs in the

TABLE 9  
RESULTS OF LEACHING SODIUM BORATE IN COLUMBIA FINE SANDY LOAM,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS  
(Harvested May 9, 1935)\*

Fraction of soil column	Check		Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.					
	10 cm H <sub>2</sub> O		0 cm H <sub>2</sub> O		2.5 cm H <sub>2</sub> O		5.0 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm
0-9.....	19	2.6	0	0.0	0	0.0	21	1.2
9-18.....	19	2.6	0	0.0	0	0.0	0	0.0
18-27.....	21	3.7	0	0.0	0	0.0	0	0.0
27-36.....	22	3.5	0	0.0	0	0.0	0	0.0
36-45.....	24	3.8	9	0.3	0	0.0	0	0.0
45-54.....	23	4.0	16	1.0	15	0.5	8	0.2
54-63.....	23	3.4	25	4.3	20	1.2	18	0.8
63-72.....	24	3.9	25	5.4†	26	5.1†	24	3.0†
72-81.....	26	5.2	30	8.9	29	6.8	30	7.6
81-90.....	28	6.7	28	8.0	28	6.1	27	7.7

Fraction of soil column	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> , 800 p.p.m.							
	10 cm H <sub>2</sub> O		20 cm H <sub>2</sub> O		40 cm H <sub>2</sub> O		80 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm
0-9.....	23	1.8	21	2.3	20	2.2	18	1.8
9-18.....	20	1.2	18	1.6	17	1.8	18	1.9
18-27.....	16	0.5	19	1.6	17	1.5	19	1.9
27-36.....	0	0.0	21	1.9	17	1.7	19	2.1†
36-45.....	0	0.0	19	1.0	18	1.3	19	2.0
45-54.....	0	0.0	8	0.2	10	1.4	19	2.0
54-63.....	0	0.0	0	0.0	19	1.4	18	1.7
63-72.....	15	0.5	0	0.0	19	1.1	18	1.9
72-81.....	24	2.0†	0	0.0	13	0.4	21	2.0
81-90.....	29	6.8	9	0.3†	9	0.3†	19	1.9

\* Average weight of plants in 10 untreated checks = 8.9 gm.

† Boron injury present to this depth.

‡ Boron injury present below this depth.

Stockton adobe clay. Chlorate was not retained at a concentration above that of the moistening solution by this soil. Borax, on the other hand, is definitely accumulated in the upper fractions from the three more concentrated solutions. As with arsenic, the quantity held (at least in this one soil) was greater with the higher concentrations of the moistening solutions.

**Leaching Studies.**—The effect of leaching upon the movement of borax within the soil columns is shown in the next set of experiments. In these tubes the initial moistening solution contained 800 p.p.m. of anhydrous borax. Seven tubes of each soil were moistened with this solution; an eighth tube was moistened with distilled water as a check. After moistening, the tubes were leached with different volumes of distilled water expressed in the tables as surface centimeters, and, after standing 48

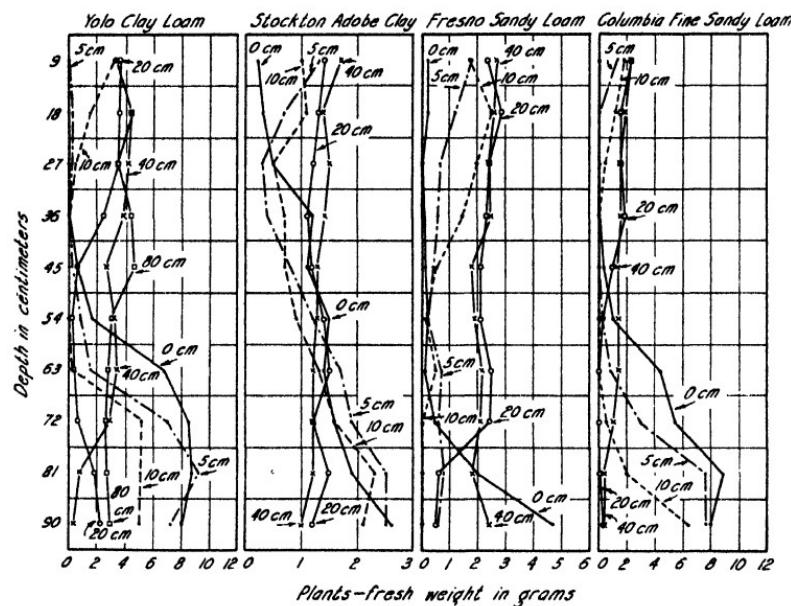


Fig. 7.—The effect of leaching upon the location of borax in soil columns as shown by crop yield.

hours, were divided into ten fractions, planted, and handled as in the previous experiments.

The data on these tests are presented in tables 6, 7, 8, and 9 and shown in the form of curves in figure 7. Comparison with the results obtained with chlorate and arsenic (17) indicate again that the borax is more firmly held than the former and less firmly than the latter. In the Yolo soil, borax was present in toxic amounts in the lower fractions of the column leached with 80 cm of water.

In the Stockton adobe clay the low toxicity of borax somewhat obscures the effects of leaching. The results would have been more clear-cut had the moistening solution contained more borax. Nevertheless, the chemical tended to remain in the top of the column and was reduced in

concentration below the limits of visible toxicity by the increased leaching instead of being displaced toward the bottom of the tube as in the other soils.

In the coarser soils the borax was readily displaced by leaching, the columns being left relatively free of chemical by the larger volumes of water.

The range of concentrations in the soil that can be tested by this method is very definitely limited by the sensitivity of the indicator plant used. Not only is the range limited, but it varies with the different soils and with the time elapsed between application and the growth of the crop. For these reasons chemical studies are needed to substantiate and extend these results. Analyses of these treated soils should give a much more accurate picture of the concentrations and the distribution of borax under the different experimental conditions.

#### PLOT TESTS WITH BORON COMPOUNDS

*Davis Plots.*—As shown by the toxicity tests reported, borax is a very toxic chemical on many soils, ranking with sodium chlorate and sodium arsenite in its ability to render the soil sterile immediately after application. It loses its toxicity rather rapidly, however, under the conditions of plant growth and, furthermore, is not firmly held in available form in the coarser soils in which it is most toxic. It is likely, therefore, to have only a limited usefulness in weed control, being effective on light soils in regions of low rainfall or upon plants especially susceptible to boron injury.

The first plots to be treated with boron compounds at Davis were established in the winter of 1933-34. They were located on the banks of an abandoned irrigation ditch on a Yolo fine sandy loam. A mixed growth of grasses and various winter annuals covered the soil at the time the applications were made. The plots were each 1 square rod in area and were treated with a number of chemicals, including borax in solution, borax in combination with sodium arsenite in solution, dry powdered borax, and mixtures of dry borax and arsenic trioxide. A few plots received colemanite, a mixed borate of sodium and calcium, somewhat less soluble than borax; others received colemanite with dry arsenic trioxide. Two plots were treated with a mixture of colemanite and sodium chlorate. The results on these plots, shown in table 10, are visual estimates of the surviving plants at the dates indicated. Check plots with arsenic and chlorate alone (14) were included, but are not reported in the table.

Evidently straight borax either dry or in solution is much less toxic in the field than sodium chlorate or arsenic. When mixed with arsenic,

TABLE 10

EFFECTS OF VARIOUS SOIL STERILANTS UPON WEED GROWTH; PLOTS AT DAVIS  
 (Applications made in December, 1933, and January, 1934)

Plot No.	Chemicals applied, pounds per square rod				Per cent weed stand on dates given		
	Chemical	Dosage	Chemical	Dosage	April 5, 1934	July 16, 1934	April 12, 1935
1	Borax in solution	2	.....	..	90	90	100
2		3	.....	..	80	90	100
3		4	.....	..	30	80	100
4		8	.....	..	40	80	95
5		12	.....	..	7	15	30
6	Borax in solution	1	Sodium acid arsenite in solution expressed in terms of $\text{As}_2\text{O}_3$	4	30	70	90
7		2		4	25	60	90
8		3		4	2	10	60
9		1		6	10	20	90
10		2		6	15	40	75
11		3		6	10	20	90
12		1		8	2	5	40
13		2		8	7	10	25
14		3		8	1	5	10
15		2		..	100	100	100
16		4		..	100	100	100
17		6		..	100	100	100
18		8		..	60	75	90
19		10		..	40	40	90
20		12		..	10	10	30
21	Borax, dry	2	Arsenic trioxide, dry	4	90	90	10
22		4		4	90	100	15
23		6		4	20	40	5
24		2		6	90	100	20
25		4		6	30	45	2
26		6		6	40	50	1
27		2		8	40	50	1
28		4		8	50	60	2
29		6		8	10	15	1
30	Colemanite, dry	4	.....	..	100	100	100
31		6	.....	..	100	100	90
32		8	.....	..	100	100	100
33		12	.....	..	75	90	60
34	Colemanite, dry	2	Arsenic trioxide, dry	4	100	100	90
35		4		4	100	100	20
36		6		4	100	100	5
37		8		4	75	75	5
38		12		4	75	100	10
39	Colemanite, dry	4	Sodium chlorate, dry	1	25	80	100
40		8		1	2	10	50

furthermore, the borax added little or nothing to the effectiveness of the treatment. In the case of the dry mixtures with arsenic the treatments were more effective the second year, probably because of a slow dissolving of the arsenic and retention in an available form in the top soil. The borax failed to improve the results of this type of application.

Arsenic, either in the form of sodium arsenite or as the dry trioxide, is very effective in soil sterilization. Its principal drawback is its poisonous nature. Sodium chlorate, though also effective, is costly, is hazardous to handle, and rapidly loses its toxicity by decomposition and leaching. For these reasons boron compounds would be preferable if they were more effective.

Probably the most promising result obtained the first season was that of the colemanite-chlorate plots listed at the bottom of table 10. These plots were relatively free of vegetation throughout the first winter and spring and for much of the second winter. The mixture of one pound of sodium chlorate with 8 pounds of colemanite would be relatively low in cost, nonpoisonous, and practically free of fire hazard.

The second season's plots were treated to test the relative effectiveness of the borate-chlorate mixtures. A portion of the same abandoned ditch was used, and applications were made in December, 1934. Results on these plots are shown in table 11.

The total seasonal rainfall during the winter of 1934-35 was 18.71 inches. At the time of application in December, 1934, 4.94 inches had fallen, leaving a difference of 13.77 inches received by the plots, much of which came in March and April. These heavy spring rains explain to a certain extent the poor results on these plots. Plots with only a scattering of weak chlorotic plants in early March later developed a fairly heavy growth. Experience indicates that the applications would have been far more effective had the spring been dry.

On the other hand, the boron compounds used in these experiments were not nearly so effective as the greenhouse results would seem to promise. Only when mixed with sodium chlorate were they satisfactory. The colemanite mixtures gave the best results under conditions at Davis.

During this same season a number of applications were made on different areas on the University Farm in an attempt to control miscellaneous weeds. These trials were made on fence lines, roadsides, graveled parking areas, graveled walks, and other places usually hoed during the spring. Most of the applications were light—from 1 to 4 pounds per square rod. Some of the treatments were repeated several times as weed growth seemed to warrant.

As the season advanced it became very evident that the treatments

TABLE 11

EFFECTS OF CHLORATE-BORATE MIXTURES UPON WEED GROWTH; PLOTS AT DAVIS  
 (Applications made in December, 1934)

Plot No.	Chemicals applied, pounds per square rod				Per cent weed stand on dates given	
	Chemical	Dosage	Chemical	Dosage	April 12, 1935	October 5, 1935*
1	Borax, dry	2	Sodium chlorate	½	70	90
2		4		1	60	80
3		6		1½	50	70
4		8		2	15	50
5		4		½	60	90
6		8		1	50	75
7		12		1½	20	40
8		16		2	10	20
9	Borax, dry	4	.....	...	100	100
10		8	.....	...	100	100
11		12	.....	...	90	100
12		16	.....	...	80	100
13	Kramer ore, dry	2	Sodium chlorate	½	80	100
14		4		1	70	90
15		6		1½	60	90
16		8		2	40	75
17		4		½	75	100
18		8		1	35	70
19		12		1½	25	50
20		16		2	15	30
21	Kramer ore, dry	4	.....	...	100	100
22		8	.....	...	100	100
23		12	.....	...	100	100
24		16	.....	...	100	100
25	Colemanite, dry	2	Sodium chlorate	½	75	100
26		4		1	30	100
27		6		1½	15	90
28		8		2	5	75
29		4		½	50	100
30		8		1	15	100
31		12		1½	5	90
32		16		2	0	50
33	Colemanite, dry	4	.....	...	100	100
34		8	.....	...	100	100
35		12	.....	...	100	100
36		16	.....	...	100	100
37	.....	..	Sodium chlorate	½	80†	100†
38	.....	..		1	70†	100†
39	.....	..		1½	60†	90†
40	.....	..		2	60†	90†

\* Winter and spring annuals on these plots were mature at the time the data were taken in April. The difference in stand in October was due to a growth of *Kickxia elatine*, a prostrate summer annual with a high salt tolerance.

† Average of 2 plots.

where two or three light applications were made were much more satisfactory than those in which the total dose was applied at one time.

Considering all of the results so far obtained with boron compounds in soil sterilization, on the fertile alluvial soils of the interior valleys of California colemanite appears to be the most effective material. Mixed with sodium chlorate at a rate of 8 parts to 1 by weight, it makes an effective sterilant if properly used. The above-mentioned experiments indicate that several light applications of the mixture through the season as the weed growth warrants are more effective than one heavy application of a like total amount. Graveled areas yield much more satisfactory results than bare soil. Dosages must be far heavier under the latter conditions. Results of using the mixture on deep-rooted perennials do not differ from those obtained by applying the chlorate which it contains alone. Borates applied to the top soil have no effect below the top few inches upon such weeds as morning-glory and creeping mallow.

*Humboldt County Plots.*—The results obtained with boron compounds in the control of Klamath weed on the more heavily leached soils of Humboldt County differ considerably from those that have just been presented.

Klamath weed (*Hypericum perforatum*), a weed pest on range lands in parts of northern California (30), is a perennial with a root system that may extend to a depth of 3 feet. It has no feed value and frequently grows in stands so dense as to crowd out desirable plants.

Experimental plot studies with soil sterilants in stands of Klamath weed in southern Humboldt County have been carried on for the past year and a half. The soil sterilants include borax, colemanite, Kramer ore (a crude sodium borate), and mixtures of these with sodium chlorate, sodium arsenite, and arsenic trioxide.

The first applications of borax and mixtures of borax with other chemicals were made near Blocksburg in March, 1934. Rainfall amounting to 8.86 inches fell between the date of application and July 1, the end of the season. These plots now have also had the 1934-35 season's rainfall of 49.23 inches—a total of 58.09 inches up to July 1, 1935. Results on these plots are given in table 12, expressed in terms of percentage stand of Klamath weed surviving the treatment. The field in which these plots are located had practically a solid stand of Klamath weed, with a small percentage of annual grasses. Removal of Klamath weed by mechanical means or by temporary soil sterilants usually results in a dense stand of annual grasses in the second year. Very little grass grew on any of the borax plots during the spring and summer of 1934. By the summer of 1935 some grass and annual weeds appeared on all plots

TABLE 12

EFFECTS OF VARIOUS CHEMICALS UPON Klamath WEED; SOIL STERILIZATION  
PLOTS AT BLOCKSBURG  
(Applications made in March, 1934)

Plot No.	Chemicals applied, pounds per square rod				Per cent weed stand on dates given		
	Chemical	Dosage	Chemical	Dosage	May 8, 1934	August 15, 1934	May 2 1935
1	Borax, dry	2	.....	..	100	90	70
2		4	.....	..	75	70	50
3		6	.....	..	60	60	40
4		8	.....	..	50	60	25
5		12	.....	..	25	15	10
6	Borax, dry	2	Arsenic trioxide, dry	4	100	80	75
7		4		4	100	90	90
8		6		4	40	30	40
9		2		8	90	70	80
10		4		8	75	75	40
11		6		8	35	40	25
12		2		12	75	40	25
13		4		12	50	25	20
14		6		12	50	30	70
15		4		1	25	2	5
16		4		2	25	2	3
17		4		3	20	1	1
18		8		1	10	3	5
19		8		2	5	1	1
20		8		3	5	1	0
21		12		1	3	1	1
22		12		2	3	0	0
23		12		3	2	0	0
24	.....	..	Sodium chlorate, dry	1	75	60	40
25	.....	..		2	60	100	100
26	.....	..		3	30	40	50
27	.....	..		4	10	3	5
28	.....	..		6	10	1	0
29	Borax in solution	1	Sodium acid arsenite in solution expressed in terms of $\text{As}_2\text{O}_3$	4	10	25	35
30		2		4	10	15	25
31		1		6	5	20	60
32		2		6	3	5	25
33		1		8	0	1	1
34		2		8	0	1	1
35	.....	..	Sodium acid arsenite in solution	4	10	25	30
36	.....	..		6	1	5	20
37	.....	..		8	1	5	15

except those treated with arsenic trioxide and sodium arsenite. All borax-treated plots had a reduced stand of grasses. Four pounds of borax per square rod reduced the stand of grasses and annual weeds by about 50 per cent; 8 pounds, by about 75 per cent.

Of this series of treatments, the mixtures of borax and sodium chlorate were the most effective against Klamath weed. The mixture of 4 pounds borax and 1 pound sodium chlorate per square rod gave satisfactory control.

The mixtures of borax with arsenic trioxide and sodium arsenite gave results no better than those of the arsenic compounds applied alone.

Another set of plots was laid out in another field in the same general locality and treated during the winter of 1934-35. Conditions of soil, topography, and plant cover were similar in the two fields. Each treatment was repeated several times between November, 1934, and April, 1935. The results of these treatments are reported in table 13. The results as stated are for Klamath weed only.

Best results with borax, both dry and in solution, were obtained from applications made about February 1. These applications had 16.22 inches of rain up to the end of the season. Eight pounds per square rod gave satisfactory control of Klamath weed under these conditions.

The dry borax-chlorate mixtures were most effective in the March 30 application. The rainfall between that date and the end of the season was 7.68 inches. This is not far from the 8.86 inches received by the series of plots treated the previous March, and the results are equally satisfactory. Again the combination treatment gave better results than either of the ingredients applied alone.

In general, results of treatments combining sodium chlorate and borax in solution are of the same order as for the corresponding dry treatments. None of the solution applications received the amount of rainfall that proved to be optimum for the dry treatments. The February 9 applications had 16.64 inches; the April 22 application but 0.84.

The November and April applications of colemanite and Kramer ore gave better results than the corresponding dry-borax treatments; but the January and February applications were not as effective as the corresponding borax application.

Combinations of colemanite with sodium chlorate, and Kramer ore with sodium chlorate were better than the corresponding combinations of sodium chlorate and borax applied on the same dates, and in general the Kramer-ore combinations appeared better than those with colemanite, though the differences may not be significant. Unfortunately, no colemanite or Kramer-ore combinations were applied on March 30,

TABLE 13

EFFECTS OF VARIOUS CHEMICALS UPON Klamath WEED; SOIL STERILIZATION  
PLOTS AT CASTERLIN, 1934-35

Plot No.	Chemicals applied, pounds per square rod				Per cent weed stand observed on Sept. 10, 1935, from applications made on dates given					
	Chemical	Dose- age	Chemical	Dose- age	Nov. 15	Dec. 8	Jan. 30	Feb. 2	Mar. 30	April 11
1	Borax, dry	4	.....	..	100	50	40	20	40	80
		8	.....	..	100	40	10	1	30	50
		12	.....	..	90	20	1	1	30	50
		16	.....	..	80	15	0	1	25	50
		20	.....	..	30	10	0	5	15	60
6	Borax in solution	2	.....	..	Nov. 22	Dec. 7	Feb. 4	Feb. 9	April 18	..
		4	.....	..	50	100	80	40	60	..
		6	.....	..	30	80	60	60	60	..
		8	.....	..	30	60	15	2	60	..
		12	.....	..	20	40	1	40	40	..
12	Borax, dry	2	Sodium chlorate, dry	1	Nov. 15	Dec. 9	Jan. 30	Feb. 2	Mar. 30	April 12
		2		2	100	25	..	..	..	..
		4		1	100	40	40	50	2	80
		4		2	70	40	50	60	2	70
		6		1	..	..	25	40	8	50
20	Borax in solution	6		2	..	..	20	60	3	20
		6		1	65	50	10	60	3	50
		8		2	40	30	10	50	3	40
		8		1	15	40	100	..	..	..
		8		2	15	30	90	..	..	..
28	Sodium chlorate, dry	4	Sodium chlorate, in solution	1	Nov. 25	Dec. 7	Feb. 5	Feb. 9	April 22	..
		4		1	10	30	60	75	70	..
		6		2	5	25	80	90	70	..
		6		1	..	..	25	70	50	..
		8		2	..	..	30	50	15	..
28	Sodium chlorate, dry	8		1	0	40	..	30	40	..
		8		2	0	20	..	30	15	..
		8		1	80	80	100	20	10	40
		8		2	75	25	80	40	15	40
		8		3	80	25	60	40	10	40
31	Sodium chlorate, dry	4	Sodium chlorate, dry	4	Nov. 16	Dec. 8	Jan. 30	Feb. 2	Mar. 30	April 11
		6		6	70	20	60	50	3	40
32	Sodium chlorate, dry	6		6	75	15	25	20	2	10

TABLE 13—*Concluded*

Plot No.	Chemicals applied, pounds per square rod				Per cent weed stand observed on Sept. 10, 1935, from applications made on dates given					
	Chemical	Dose- age	Chemical	Dose- age						
33	Sodium chlorate in solution	...	Sodium chlorate in solution	1	50	100	25	50	75	..
34		...		2	70	70	30	35	50	..
35		...		3	40	60	40	40	20	..
36		...		4	20	40	40	40	15	..
37		...		5	50	40	50	50	20	..
					Nov. 20	Feb. 3	April 11			
38	Colemanite, dry	4	Colemanite, dry	..	80	90	70	..	..	..
39		8		..	80	50	15	..	..	..
40		12		..	80	20	15	..	..	..
41		16		..	30	2	15	..	..	..
42		20		..	30	5	10	..	..	..
					Nov. 20	Feb. 3	April 11			
43	Kramer ore, dry	4	Kramer ore, dry	..	70	50	40	..	..	..
44		8		..	70	40	15	..	..	..
45		12		..	30	10	15	..	..	..
46		16		..	5	2	10	..	..	..
47		20		..	5	20	10	..	..	..
					Nov. 20	Feb. 3	April 17			
48	Colemanite, dry	2	Sodium chlorate, dry	1	75	..	..	..	..	..
49		2		2	75	..	..	..	..	..
50		4		1	80	30	15	..	..	..
51		4		2	35	50	5	..	..	..
52		6		1	..	60	5	..	..	..
53		6		2	..	50	5	..	..	..
54		8		1	50	50	8	..	..	..
55		8		2	30	40	3	..	..	..
					Nov. 20	Feb. 3	April 18			
56	Kramer ore, dry	2	Sodium chlorate, dry	1	70	..	..	..	..	..
57		2		2	50	..	..	..	..	..
58		4		1	50	15	10	..	..	..
59		4		2	30	60	5	..	..	..
60		6		1	..	30	8	..	..	..
61		6		2	..	30	5	..	..	..
62		8		1	50	15	3	..	..	..
63		8		2	50	10	2	..	..	..

the date that gave the best results with the borax combinations. The applications of April 17 and 18 received but 0.84 inch of rain; and though the results are significantly better than from the ores alone or chlorate alone, the treatments would probably have been still more effective had they received more rainfall.

#### DISCUSSION AND SUMMARY

Although boron compounds are extremely toxic to some plants and may reduce growth and produce characteristic symptoms when present in relatively low concentrations in the soil, in chemical weed control they will find only limited use. The high toxicity indicated in the greenhouse tests is less evident in the field. These compounds, furthermore, are not retained in the soil against the leaching power of moving water to the same extent as arsenic. For complete sterilization, in consequence, dosages must be heavy and applications frequent. The discrepancy between the toxicity expressed in the greenhouse tests and that observed on field plots seems somewhat anomalous until one considers the time effect. As shown by figures 1, 2, 3, and 4, borax loses rapidly in toxicity when held in a given mass of soil. In the field this action is superimposed on the effects of the loss by leaching from the surface soil, and together these losses reduce the effective concentration until seedlings are permitted to develop. When these seedlings are of a tolerant species they grow, and the plots become reinfested. Evidently, therefore, the use of boron in soil sterilization will be limited both by the species existent on the areas and by the amount and distribution of the seasonal rains.

The results on the Davis plots are given in terms of the effects of boron compounds upon growth of the common winter annuals of that region. Most of these plants, adapted to growing in regions of medium to low rainfall, thrive in soils containing a fairly high level of soluble salts. They are all fairly tolerant of boron, and relatively high concentrations in the soil solution are required to kill them. Klamath weed (*Hypericum perforatum*), a native of northern Europe, grows only in regions of medium to high rainfall, where soils are heavily leached and contain relatively little soluble material. Klamath weed, therefore, is injured by high concentrations of salts in the soil solution and is highly susceptible to boron poisoning. Therefore boron compounds should be useful in controlling Klamath weed, especially when combined with sodium chlorate to increase the toxicity. Their low solubility should result in some residual effect, reducing the probability of reinfestation by seedlings. Their nonpoisonous and fire-deterrant properties should appreciably reduce the hazard over that of sodium chlorate or arsenic.

Against more tolerant plants and in regions of fertile, recent alluvial soils the use of boron compounds seems limited to the coarser soils or to graveled areas; and where rainfall exceeds 10 inches annually, applications will be needed one or more times each year.

Again it should be emphasized that boron compounds must be used with caution close to ornamental plants, around orchards or groves of such susceptible species as citrus and walnuts, and in regions where there is a possibility of contamination of water supplies used for irrigation.

With these obvious drawbacks, however, the combination of sodium chlorate with some one of the cheap boron ores should find wide use in such places as school grounds, parking areas, airports, and graveled walks and driveways, where a nonpoisonous soil sterilant is desirable.

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SOME EFFECTS OF THALLIUM SULFATE  
UPON SOILS

A. S. CRAFTS



# SOME EFFECTS OF THALLIUM SULFATE UPON SOILS<sup>1</sup>

A. S. CRAFTS<sup>2</sup>

## INTRODUCTION

WITH THE INCREASING USE of poisons for pest control in agriculture, new and little-known chemicals are frequently introduced. The ultimate effects of these reagents upon soils and crops may present serious problems, and the continued use of certain of them cannot be safely recommended until their long-time behavior is understood.

Brooks (1)<sup>4</sup> has warned of the possible sterilization effects of thallium sulfate used in rodent control, and McCool (4) has confirmed the highly toxic nature of this chemical in soils.

In pest control, toxicity is of eminent importance; and in weed work, soil effects are of special interest. Although thallium compounds are too expensive to be practical in weed control, their behavior in soils characterizes a certain type of toxic materials. A study of their reactions should contribute to our general information.

A preliminary report on work done on the problem of thallium toxicity in California soils has been published (2). The method used in toxicity studies, as already described by the author (3) in a previous paper, consists principally in pot-culture tests using 500-gram lots of soils in No. 2 cans as the culture media. The chemicals to be tested are applied to the soils in various ways, and their effects upon indicator plants (Kanota oats) are measured by recording height and fresh weight of the latter after a 30-day growth period. The details of the individual tests with thallium will become apparent in the following pages.

<sup>1</sup> Received for publication May 8, 1936.

<sup>2</sup> This investigation was undertaken at the request of a special committee appointed in the University of California in 1932 to study problems involved in rodent and wild-life control. The use of chemicals, including thallium sulfate, for rodent control was studied by this committee. Certain claims had been made as to the possible or probable sterilizing effect of thallium salts if distributed in connection with rodent control. The literature then available did not provide satisfactory answers to these claims. The Division of Botany of the College of Agriculture was asked by the committee to conduct a study of the effect of thallium sulfate on soils. Dr. T. I. Storer of the Zoology Division of the College of Agriculture, who was a member of the above committee, coöperated in the planning and execution of the experiments. The paper presented herewith incorporates the results of this study. Dr. Crafts found, however, that certain general principles with respect to the effect of salts of heavy metals on soils could be elucidated by use of thallium sulfate, and the studies were therefore carried farther than necessary to provide an answer to the original request.—C. B. Hutchison, Director of the Agricultural Experiment Station.

<sup>3</sup> Assistant Professor of Botany and Assistant Botanist in the Experiment Station.

<sup>4</sup> Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

## EXPERIMENTS

*Toxicity Studies.*—Two toxicity series have been run with thallium sulfate. The first, a short concentration series in Yolo clay loam, having been cropped twelve times, gives a picture of the effects of time and re-

TABLE 1

TOXICITY OF THALLIUM SULFATE UPON SUCCEEDING CROPS IN YOLO CLAY LOAM,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS\*

Tl <sub>2</sub> SO <sub>4</sub> Amount in air-dry soil	First run harvested Oct. 29, 1932		Second run harvested Jan. 22, 1933		Third run harvested May 6, 1933		Fourth run harvested Nov. 18, 1933		Fifth run harvested Jan. 14, 1934		Sixth run harvested Mar. 10, 1934	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
p.p.m.	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
15.....	28	9.6	31	7.5	35	8.8	25	6.6	29	5.2	35	8.1
30.....	28	8.6	28	6.1	35	8.7	26	6.6	30	5.8	34	9.6
60.....	25	5.1	20	3.3	31	5.6	25	6.9	30	4.6	35	9.2
120.....	20	1.8	13	1.8	23	2.9	23	2.8	22	3.2	24	3.8
180.....	15	1.0	8	1.0	18	1.7	18	1.8	16	2.2	17	2.0
240.....	10	0.5	6	0.4	10	0.6	15	1.1	12	1.5	11	1.3
300.....	7	0.4	5	0.3	8	0.5	11	0.8	12	1.2	7	0.5
375.....	4	0.2	4	0.3	7	0.4	8	0.6	11	0.9	6	0.3
450.....	3	0.1	3	0.2	5	0.2	7	0.5	10	0.6	6	0.1
600.....	3	0.1	3	0.2	3	0.2	8	0.4	8	0.5	6	0.1
Check.....	28	9.4	28	8.0	33	8.3	24	6.2	30	5.3	32	8.6
Tl <sub>2</sub> SO <sub>4</sub> amount in air-dry soil	Seventh run† harvested July 23, 1934		Eighth run harvested Oct. 27, 1934		Ninth run harvested Mar. 1, 1935		Tenth run harvested Nov. 20, 1935		Eleventh run harvested Feb. 3, 1936		Twelfth run harvested April 17, 1936	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
p.p.m.	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
15.....	12	3.7	28	4.2	28	5.2	23	5.6	29	6.8	31	9.2
30.....	12	3.6	25	3.3	29	5.5	22	5.3	28	5.9	32	8.1
60.....	12	4.0	28	4.4	28	5.6	24	5.9	29	5.8	29	8.4
120.....	12	3.6	26	3.4	24	3.3	22	3.5	24	3.3	33	7.6
180.....	9	2.1	19	1.6	16	1.5	16	1.6	18	1.4	24	3.2
240.....	7	1.1	14	1.0	11	0.8	10	1.0	14	0.8	17	1.6
300.....	5	0.5	9	0.5	8	0.6	9	0.4	12	0.4	10	0.6
375.....	4	0.4	7	0.3	7	0.5	8	0.3	14	0.3	8	0.2
450.....	4	0.2	7	0.2	6	0.4	8	0.2	8	0.2	7	0.1
600.....	4	0.1	6	0.2	6	0.3	7	0.2	9	0.2	7	0.1
Check.....	12	3.4	27	4.2	26	4.8	23	5.0	29	6.6	32	9.8

\* All cultures run in triplicate; checks replicated six times. All values are averages of the replicates.

† Run No. 7 was conducted out of doors at Berkeley; all others were conducted in the greenhouse at Davis.

peated cropping upon the availability of this chemical. The results of the first, third, and fifth crops in comparison with crops with other sterilants have been reported (3). Table 1 gives the complete data on this experiment in terms of plant growth, which, of course, varies inversely

with toxicity. In this table rapid changes in toxicity are indicated by sudden changes in fresh weight, as between 60 p.p.m. and 120 p.p.m. in the first run.

The differences in toxicity evident in these results indicate a drop to about one-half the initial toxicity shown by the first run; then values tend to fluctuate in response to changes in light, temperature, and humidity; they follow no definite trend. Low values are shown in run 7,

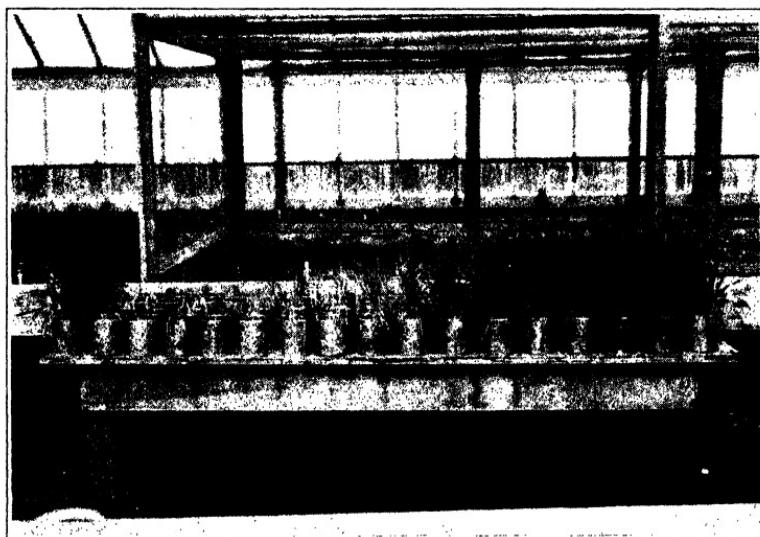


Fig. 1.—A concentration series with thallium sulfate in Yolo clay loam on test in the greenhouse. One row of checks is located at each end of the series. The concentration increases from right to left. Photograph taken on January 19, 1933, showing the first run reported in table 2.

probably because that run was conducted out of doors in Berkeley under conditions of high light intensity and high humidity. The other eleven runs were made in the greenhouse at Davis under conditions of low humidity during a large portion of the year.

The second toxicity experiment was conducted with four California soils, using thallium sulfate alone. The air-dry soils were moistened with solutions containing thallium sulfate in the concentrations given in the first column of table 2. Each culture was replicated five times, and the complete set run twice. Figure 1 shows the first run in the Yolo clay loam. Table 2 gives the yield data.

The differences in toxicity of thallium sulfate in the four soils is striking. They do not correlate well with water-holding capacities nor with the concentrations based on air-dry weights. No significant changes were

TABLE 2

TOXICITY OF THALLIUM SULFATE IN FOUR TYPES OF CALIFORNIA SOILS,  
AS SHOWN BY GROWTH OF INDICATOR PLANTS

Tl <sub>2</sub> SO <sub>4</sub> in moistening solution, p.p.m.	Yolo clay loam			Fresno sandy loam			Stockton adobe clay			Columbia fine sandy loam		
	Tl <sub>2</sub> SO <sub>4</sub> in air-dry soil	Ht.	Wt.	Tl <sub>2</sub> SO <sub>4</sub> in air-dry soil	Ht.	Wt.	Tl <sub>2</sub> SO <sub>4</sub> in air-dry soil	Ht.	Wt.	Tl <sub>2</sub> SO <sub>4</sub> in air-dry soil	Ht.	Wt.

First run, harvested January 29, 1933

	p.p.m.	cm	gm	p.p.m.	cm	gm	p.p.m.	cm	gm	p.p.m.	cm	gm
25.....	7.5	32.5	10.6	3.3	20.6	3.4	5.8	12.7	1.5	3.7	21.2	5.4
50.....	15.0	30.7	10.0	6.5	20.1	3.8	11.7	12.7	1.4	7.5	22.4	5.7
75.....	22.5	29.5	9.4	9.8	19.6	3.0	17.5	12.5	1.3	11.2	21.4	5.4
100.....	30.0	28.4	9.1	13.1	18.3	3.0	23.3	9.5	1.0	15.0	21.2	5.6
150.....	45.0	27.0	7.8	19.6	18.1	3.0	35.0	9.7	1.1	22.5	20.3	5.1
200.....	60.0	24.0	5.1	26.2	16.5	2.6	46.6	9.0	1.0	30.0	20.1	5.2
275.....	82.5	21.2	3.4	36.0	14.0	2.0	64.2	7.5	0.5	41.2	20.5	4.6
350.....	105.0	18.8	2.5	45.8	12.8	1.6	81.7	7.7	0.6	52.5	19.0	4.3
450.....	135.0	13.7	1.5	58.9	12.0	1.5	105.0	5.3	0.5	67.5	18.3	4.3
550.....	165.0	11.9	1.8	72.0	9.2	1.0	128.5	5.1	0.4	82.5	16.3	3.1
650.....	195.0	12.5	1.3	85.0	7.9	0.9	151.8	5.0	0.4	97.5	15.2	2.6
800.....	240.0	4.8	0.5	104.6	5.1	0.7	186.8	4.8	0.3	120.0	12.5	1.9
1,000.....	300.0	6.0	0.9	131.0	3.8	0.3	233.3	4.4	0.3	150.0	10.4	1.7
1,500.....	450.0	5.0	0.5	196.1	3.0	0.2	350.0	3.9	0.3	225.0	6.5	1.1
2,000.....	600.0	5.0	0.6	262.0	2.5	0.2	467.0	3.5	0.3	300.0	5.8	0.9
Check.....	.....	31.5	10.0	.....	19.1	3.4	.....	12.5	2.0	.....	21.4	5.7
Check.....	.....	28.8	10.4	.....	20.5	3.8	.....	12.5	2.1	.....	21.5	6.2

Second run, harvested April 17, 1933

	p.p.m.	cm	gm									
25.....	7.5	30.5	8.8	3.3	18.3	3.2	5.8	15.1	2.6	3.7	25.4	3.4
50.....	15.0	31.3	8.6	6.5	17.8	2.9	11.7	17.0	2.7	7.5	25.4	3.5
75.....	22.5	31.0	9.0	9.8	17.3	2.5	17.5	17.2	2.6	11.2	25.0	3.8
100.....	30.0	31.5	8.7	13.1	14.0	1.9	23.3	16.6	2.4	15.0	24.4	3.5
150.....	45.0	28.3	7.7	19.6	12.2	1.3	35.0	15.4	2.0	22.5	23.6	3.2
200.....	60.0	16.2	6.7	26.2	10.2	0.7	46.6	13.7	1.8	30.0	23.6	3.2
275.....	82.5	13.2	4.6	36.0	9.9	0.5	64.2	10.1	1.3	41.2	22.1	3.0
350.....	105.0	20.0	3.2	45.8	5.1	0.1	81.7	9.4	0.5	52.5	20.1	2.5
450.....	135.0	15.7	1.8	58.9	4.0	0.1	105.0	8.7	0.3	67.5	18.0	2.0
550.....	165.0	12.0	0.7	72.0	3.7	0.1	128.5	7.6	0.2	82.5	14.7	1.6
650.....	195.0	10.3	0.6	85.0	3.0	0.1	151.8	7.1	0.1	97.5	11.5	1.1
800.....	240.0	9.0	0.2	104.6	2.5	0.1	186.8	6.4	0.1	120.0	9.1	0.9
1,000.....	300.0	6.2	0.2	131.0	2.5	0.1	233.3	5.5	0.1	150.0	6.4	0.8
1,500.....	450.0	4.4	0.2	196.1	2.0	0.1	350.0	5.0	0.1	225.0	5.1	0.2
2,000.....	600.0	3.9	0.1	262.0	2.0	0.1	467.0	5.0	0.1	300.0	5.1	0.2
Check.....	.....	28.0	8.0	.....	17.8	3.2	.....	14.8	2.5	.....	26.0	3.8
Check.....	.....	30.5	9.0	.....	17.6	3.0	.....	14.8	2.8	.....	22.9	3.8

shown in the second run. This chemical shows extreme and persistent toxicity in soils of low fertility.

The response of the cereals to thallium sulfate in toxic concentrations is characteristic. Whereas chlorates and arsenic in toxic doses retard growth of the complete embryo, thallium checks the development of the shoot but has little effect on the coleoptile. Table 3 gives the relative development of shoots and coleoptiles of the oat seedlings 5 days after planting the second crop in two of the soils reported in table 2.

TABLE 3

RELATIVE DEVELOPMENT OF SHOOTS AND COLEOPTILES OF OATS GROWN IN THE SECOND RUN ON THALLIUM-TREATED SOILS, 5 DAYS AFTER PLANTING

Soil type	Plant part	Tl <sub>2</sub> SO <sub>4</sub> in moistening solution, p.p.m.												Check
		100	150	200	275	350	450	550	650	800	1,000	1,500	2,000	
Yolo clay loam	Shoots	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm	cm
	Coleoptiles	15.0	15.0	15.0	15.0	15.0	12.5	12.5	10.0	7.5	4.0	3.2	2.5	15.0
Fresno sandy loam	Shoots	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.5	4.0	4.0	4.0	5.0
	Coleoptiles	15.0	12.5	11.5	10.0	7.5	5.0	4.0	3.2	2.5	0.7	0.0	0.0	15.0

In the higher concentrations the shoots grow very slowly, sometimes not protruding beyond the tips of the coleoptiles. In these extreme cases the seedlings usually die after two or three weeks. Where the chemical is less concentrated, the shoots continue growth; but with the development of the leaf blades they become chlorotic and weak. As shown in table 2, only the plants in the very low concentrations attained anything like normal development. Plants that make a perfectly normal start, as, for instance, those reported on the left in table 3, may subsequently show chlorosis, weakening, and decline. This fact is illustrated in table 2 where, in the second run in Fresno sandy loam, the plants in the culture containing 100 p.p.m. of Tl<sub>2</sub>SO<sub>4</sub> in the soil solution made but little more than half the normal growth. These differences would be even greater if the plants were grown for a longer period.

*Soil-Tube Tests.*—The distribution of a sterilant within the soil after its application to the surface depends primarily upon the fixing power of the soil for the chemical. This property was studied by the soil-tube method previously described (3). Briefly, this consisted in slowly moistening columns of air-dry soil with Tl<sub>2</sub>SO<sub>4</sub> solutions, dividing the columns each into 9 fractions of equal weight and approximately 10 cm in height, and growing oats upon the fractions. Results are shown in table 4.

TABLE 4

## FIXING POWER OF CALIFORNIA SOILS FOR THALLIUM SULFATE AS SHOWN BY GROWTH OF INDICATOR PLANTS IN FRACTIONS OF THE TREATED SOIL COLUMNS\*

Soil type	Fraction of column	Concentration of thallium sulfate in the moistening solution											
		H <sub>2</sub> O check		25 p.p.m.		50 p.p.m.		100 p.p.m.		200 p.p.m.		400 p.p.m.	
		Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
Yolo clay loam harvested Jan. 24, 1933	cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
	0-10	19	3.2	17	2.0	13	1.1	13	0.7	10	0.6	4	0.4
	10-20	21	3.5	21	3.7	19	3.5	19	3.3	20	3.1	20	2.8
	20-30	20	3.7	19	3.0	20	3.5	19	3.2	20	3.4	19	3.6
	30-40	20	3.6	21	3.5	20	4.2	20	3.3	23	3.8	19	3.5
	40-50	21	3.6	20	3.3	20	3.8	19	3.5	20	3.2	19	4.2
	50-60	20	3.4	20	3.3	20	3.9	19	3.3	20	3.4	19	3.2
	60-70	22	3.9	21	3.3	30	9.2	21	4.2	27	7.0	28	8.0
	70-80	29	9.2	29	9.6	30	9.9	30	9.4	28	9.3	30	10.0
	80-90	27	7.5	27	6.5	31	10.0	31	7.9	29	7.6	23	6.3
Fresno sandy loam harvested Feb. 19, 1933	0-10	16	3.0	7	0.8	5	0.7	3	0.3	3	0.2	2	0.1
	10-20	17	2.9	15	2.5	15	2.8	15	2.9	15	2.9	12	2.6
	20-30	18	3.3	18	3.5	16	3.0	17	3.1	16	3.1	13	3.1
	30-40	18	3.0	18	3.2	18	3.2	17	3.4	17	3.0	15	3.1
	40-50	18	3.4	17	2.9	18	3.3	18	3.3	17	3.2	17	3.2
	50-60	17	3.2	18	3.2	18	3.6	16	3.0	17	3.1	19	4.0
	60-70	17	3.1	18	3.3	17	3.1	17	3.1	17	3.3	17	3.0
	70-80	17	3.2	17	3.2	17	3.2	17	3.2	17	3.2	17	3.1
	80-90	18	3.4	18	3.6	21	5.6	17	3.3	17	3.6	17	3.4
Stockton adobe clay harvested March 5, 1933	0-10	13	1.9	8	0.4	8	0.1	6	0.1	6	0.1	5	0.1
	10-20	14	2.0	13	2.1	13	2.0	15	2.2	14	1.9	14	1.7
	20-30	14	2.0	13	2.0	13	1.9	14	2.0	15	2.2	14	1.9
	30-40	14	2.1	13	2.0	13	2.0	14	2.1	14	2.1	14	1.9
	40-50	13	2.0	13	2.1	13	1.8	13	2.0	14	2.0	13	1.7
	50-60	14	2.2	13	1.9	14	2.2	14	2.2	14	2.0	14	1.6
	60-70	14	2.2	13	1.9	14	2.1	14	2.3	14	2.2	14	1.5
	70-80	14	2.3	14	2.3	14	2.3	14	2.2	13	2.0	13	2.1
	80-90	13	2.1	13	2.2	13	1.9	14	2.2	13	2.1	13	1.7
Columbia fine sandy loam harvested March 24, 1933	0-10	19	3.4	18	2.7	13	2.5	8	0.1	5	0.1	5	0.1
	10-20	20	3.3	20	3.3	19	3.6	20	3.0	20	3.1	18	3.2
	20-30	20	3.5	19	3.4	20	3.6	20	3.5	30	3.8	20	3.5
	30-40	21	3.7	20	3.6	20	4.0	20	3.6	20	3.8	20	3.6
	40-50	20	3.9	20	3.9	20	3.7	21	3.9	21	4.0	21	3.9
	50-60	20	3.7	20	3.8	21	3.9	20	4.0	20	3.8	20	3.8
	60-70	20	3.9	20	3.6	21	4.2	21	4.2	20	3.7	20	3.7
	70-80	23	5.8	21	5.8	23	4.7	25	6.6	23	4.6	25	7.2
	80-90	29	7.5	30	6.8	28	7.1	30	8.5	30	8.9	28	6.2

\* Average fresh weight of plants in 12 untreated checks: Yolo clay loam, 9.0 grams; Fresno sandy loam, 3.3 grams; Stockton adobe clay, 2.0 grams; Columbia fine sandy loam, 5.3 grams.

Apparently all the chemical was held in the top 10 cm of soil in all these tests. In the more fertile soils, namely the Yolo clay loam and the Columbia fine sandy loam, there was appreciable leaching of soil nutrients. Since the moisture was so measured that it did not quite completely wet the soil, these nutrients were present in the lower portions of the soil columns and stimulated the plants in these fractions. In the Fresno sandy loam and Stockton adobe clay no appreciable leaching of nutrients

TABLE 5  
FIXING POWER OF YOLO CLAY LOAM FOR THALLIUM SULFATE\*  
(Harvested April 10, 1933)

Fraction† of column	Concentration of $Tl_2SO_4$ in moistening solution, p.p.m.								Fraction‡ of column	Concentration of $Tl_2SO_4$ in moistening solution, p.p.m.		
	25		100		500		1,000			1,000		
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.		Ht.	Wt.	
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	cm	gm	
0.0-2.5	18	1.7	5	0.1	3	0.1	1	0.0	0.0-1.2	1	0.0	
2.5-5.0	23	6.2	22	6.5	23	6.9	18	2.3	1.2-2.5	1	0.0	
5.0-7.5	24	6.6	24	6.1	25	6.4	23	6.7	2.5-3.7	8	0.2	
7.5-10.0	25	7.2	25	6.9	25	6.3	25	7.0	3.7-5.0	23	7.6	
10.0-12.5	25	6.8	25	7.3	25	7.1	28	7.1	5.0-6.2	25	7.8	
12.5-15.0	25	6.8	26	7.3	28	7.5	26	6.7	6.2-7.5	28	9.2	
15.0-17.5	25	6.5	25	7.2	29	7.8	28	7.6	7.5-8.7	28	8.5	
17.5-20.0	25	6.9	26	7.3	28	7.6	26	6.9	8.7-10.0	28	9.1	
20.0-22.5	25	7.1	28	7.4	28	7.0	26	6.9	10.0-11.2	28	9.3	
22.5-25.0	26	7.3	28	7.6	28	7.1	27	7.2	11.2-12.5	27	8.7	
25.0-27.5	28	7.6	28	8.1	27	7.2	26	7.1	12.5-15.0	27	8.2	
27.5-30.0	25	6.7	28	7.4	27	7.0	27	6.8	15.0-17.5	27	9.0	

\* Average fresh weight of 10 untreated checks = 9.8 grams.

† Each fraction mixed with 375 grams air-dry soil and moistened with 112.5 cc tap water so that the culture has a thallium concentration one-quarter that of the moistening solution.

‡ Each fraction mixed with air-dry soil and tap water to make 650 grams of moistened soil per culture.

occurred; growth was uniform in all the lower fractions. The checks in column 3, table 4 were simply cultures in newly moistened soils.

Thallium sulfate is apparently held very firmly in all these soils. In none was there any evidence that the capacity of the soil for the sterilant was exceeded. The top 10 cm (table 4) held all the chemical applied to each tube. Since the quantity of moistening solution applied was just short of enough to wet the soil to the full depth, and this top fraction is one-ninth of the total depth, approximately one-ninth of the water would be held in this top fraction. Where the moistening solution contained 400 p.p.m., the concentration in the top fraction would therefore be 3,600 p.p.m. This was about 1,080 p.p.m. on the air-dry soil basis in the Yolo clay loam, 471 in the Fresno sandy loam, 840 in the Stockton adobe clay, and 540 in the Columbia fine sandy loam.

To ascertain the depth to which the chemical was penetrating, a series of 5 tubes was run using Yolo clay loam and fractionating into layers approximately 2.5 cm thick (table 5). These 2.5 cm portions were mixed in each case with 375 grams of air-dry soil. The mixtures were then placed in cans and moistened with 112.5 cc of tap water. In other words, the soil in each fraction was diluted with three parts of untreated soil. Consequently, in table 5 the concentrations of thallium sulfate expressed in terms of the moistening solution must be divided by 4 to give the actual values for the cultures. In the fifth tube the column was fractionated at each 1.2 cm, and the portions were made up to 650 grams of moistened soil by adding 7 parts of dry soil and wetting with tap water.

These results of what constitutes a more detailed study of the fixing of thallium sulfate by Yolo clay loam conclusively show that this chemical in solutions up to 500 p.p.m. in concentration will be all taken up in the top 2.5 em of this soil from a volume sufficient to wet a 90-cm column (table 5). When the concentration reaches 1,000 p.p.m., the top 3.7 cm will hold the chemical. If the concentration in this top 3.7 cm were uniform, it would be 24,000 p.p.m. on the basis of the soil moisture or 7,200 p.p.m. in the air-dry soil. Since the growth was somewhat greater in the third culture, the concentration was probably greater in the top 2.5 cm. The capacity of Yolo clay loam to hold thallium sulfate may be safely estimated at around 10,000 p.p.m. on the dry-soil basis. In this soil, therefore, thallium is held up to a concentration of 1 per cent of its weight against the leaching effects of moving water in a form available to plants. Such a chemical, if applied to the soil, would remain in a relatively shallow layer for a considerable period, which renders it sterile to plant growth.

Most agricultural soils in California are subject to considerable moisture movement. The resistance of a chemical sterilant to the leaching effects of rains or irrigation is of vital importance. Thallium sulfate resists leaching to a marked degree, as shown by the following experiments. Tubes of air-dry soils, moistened with solutions containing 100 p.p.m. and 200 p.p.m. of thallium sulfate, were leached with varying amounts of distilled water. When the leaching was finished they were allowed to come to equilibrium by standing with their lowermost layers in contact with air-dry soil until the moisture stopped moving. They were then fractionated, planted with oats, and at the end of a 30-day period the oats were harvested as in the previous tests. Tables 6, 7, 8, and 9 present the data on these experiments.

With the Yolo clay loam, as the volume of water used in leaching becomes great enough to carry the soil nutrients out of the tube, the

TABLE 6

## RESULTS OF LEACHING THALLIUM SULFATE IN YOLO CLAY LOAM WITH DIFFERENT DEPTHS OF WATER, AS SHOWN BY GROWTH OF INDICATOR PLANTS\*

Tl<sub>2</sub>SO<sub>4</sub>, 100 p.p.m. in moistening solution, cultures harvested January 27, 1933

Depth cm	Leached with 5 cm H <sub>2</sub> O		Leached with 10 cm H <sub>2</sub> O		Leached with 15 cm H <sub>2</sub> O		Leached with 20 cm H <sub>2</sub> O		Leached with 25 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
0-10.....	7	1.1	5	0.6	7	1.6	10	0.8	13	2.2
10-20.....	19	3.9	20	3.4	20	3.6	18	3.2	19	3.5
20-30.....	19	3.8	21	4.4	20	3.7	19	3.4	20	4.3
30-40.....	20	4.2	22	3.8	20	3.6	19	3.1	19	3.8
40-50.....	22	3.6	22	3.5	19	3.3	19	3.5	19	3.8
50-60.....	22	3.9	22	3.5	19	3.5	19	3.6	20	4.7
60-70.....	24	5.8	21	3.4	18	2.9	18	3.2	18	3.5
70-80.....	30	10.0	20	3.4	18	3.1	19	3.4	19	3.8
80-90.....	30	10.9	20	11.0	19	3.7	18	3.3	18	3.5

Tl<sub>2</sub>SO<sub>4</sub>, 200 p.p.m. in moistening solution, cultures harvested February 7, 1933

Depth	Leached with 37.5 cm H <sub>2</sub> O		Leached with 50 cm H <sub>2</sub> O		Leached with 75 cm H <sub>2</sub> O		Leached with 125 cm H <sub>2</sub> O		Leached with 200 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
0-10.....	5	0.5	5	0.5	5	0.4	5	0.5	5	0.4
10-20.....	22	4.0	20	4.2	22	4.5	18	4.2	17	3.0
20-30.....	22	4.3	19	3.8	23	5.2	20	4.7	18	4.0
30-40.....	22	4.5	20	4.0	24	5.0	20	4.8	19	4.1
40-50.....	22	4.7	20	3.9	23	5.2	20	4.2	19	3.8
50-60.....	23	5.2	21	4.4	22	3.6	20	4.2	18	3.2
60-70.....	20	3.9	22	4.3	22	3.7	18	3.3	17	3.0
70-80.....	20	3.9	20	4.4	22	4.0	17	3.0	17	3.2
80-90.....	20	4.1	20	4.0	22	4.6	15	2.8	15	2.6

Check tubes moistened with water, cultures harvested February 7, 1933

Depth	Leached with 37.5 cm H <sub>2</sub> O		Leached with 50 cm H <sub>2</sub> O		Leached with 75 cm H <sub>2</sub> O		Leached with 125 cm H <sub>2</sub> O		Leached with 200 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
0-10.....	19	4.0	18	3.4	19	3.8	18	3.5	17	3.0
10-20.....	19	3.8	19	3.7	20	4.3	18	3.7	17	3.1
20-30.....	20	4.1	20	4.0	20	4.2	18	3.5	17	3.2
30-40.....	19	3.7	23	4.9	20	4.6	19	4.1	18	4.0
40-50.....	19	4.0	21	4.2	19	4.0	17	3.1	19	3.4
50-60.....	20	4.3	22	4.5	20	4.4	17	3.2	20	4.7
60-70.....	19	4.3	19	3.8	19	3.3	17	3.1	19	3.7
70-80.....	19	3.7	19	3.4	20	4.0	18	3.3	21	4.5
80-90.....	19	4.3	19	3.5	19	3.9	19	3.5	19	3.6

\* Average weight of plants in 30 untreated checks = 11.6 gm.

TABLE 7

## RESULTS OF LEACHING THALLIUM SULFATE IN FRESNO SANDY LOAM WITH DIFFERENT DEPTHS OF WATER, AS SHOWN BY GROWTH OF INDICATOR PLANTS\*

Tl<sub>2</sub>SO<sub>4</sub>, 100 p.p.m. in moistening solution, cultures harvested February 16, 1933

Depth	Leached with 5 cm H <sub>2</sub> O		Leached with 10 cm H <sub>2</sub> O		Leached with 15 cm H <sub>2</sub> O		Leached with 20 cm H <sub>2</sub> O		Leached with 25 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-10.....	1	0.1	1	0.1	1	0.2	1	0.2	1	0.1
10-20.....	14	2.6	14	2.3	13	2.4	14	2.3	13	2.1
20-30.....	14	2.6	14	2.4	13	2.2	13	2.5	15	2.8
30-40.....	13	2.5	14	2.6	13	2.4	14	2.8	14	2.3
40-50.....	14	2.7	14	2.6	13	2.5	14	2.5	14	2.7
50-60.....	14	2.9	14	2.8	14	2.7	15	2.6	14	2.4
60-70.....	14	2.7	14	2.8	14	2.6	14	2.5	14	2.8
70-80.....	14	2.8	14	2.9	14	2.4	14	2.4	14	2.7
80-90.....	14	3.1	15	2.8	14	2.4	15	2.5	14	2.3

Tl<sub>2</sub>SO<sub>4</sub>, 200 p.p.m. in moistening solution, cultures harvested March 20, 1933

Depth	Leached with 37.5 cm H <sub>2</sub> O		Leached with 50 cm H <sub>2</sub> O		Leached with 75 cm H <sub>2</sub> O		Leached with 125 cm H <sub>2</sub> O		Leached with 200 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-10.....	3	0.1	3	0.1	2	0.1	1	0.1	2	0.1
10-20.....	15	2.7	15	2.7	15	2.7	14	2.5	14	2.4
20-30.....	14	2.5	15	2.8	15	2.7	15	2.6	15	2.8
30-40.....	15	2.6	15	2.8	15	2.8	15	2.7	15	2.9
40-50.....	16	2.8	16	2.8	16	2.7	15	3.2	16	2.9
50-60.....	14	2.6	16	2.9	14	2.8	15	2.9	16	3.0
60-70.....	14	2.8	15	2.9	14	2.7	14	2.9	15	2.8
70-80.....	15	2.6	15	2.9	15	2.5	15	2.8	16	2.7
80-90.....	15	2.8	15	2.7	15	2.3	14	2.6	18	3.3

Check tubes moistened with water, cultures harvested March 20, 1933

Depth	Leached with 37.5 cm H <sub>2</sub> O		Leached with 50 cm H <sub>2</sub> O		Leached with 75 cm H <sub>2</sub> O		Leached with 125 cm H <sub>2</sub> O		Leached with 200 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-10.....	15	2.7	16	2.5	15	2.7	13	2.4	14	2.2
10-20.....	16	2.9	16	2.7	15	2.7	15	2.7	15	2.5
20-30.....	15	2.9	17	3.0	15	2.8	15	2.9	15	2.7
30-40.....	15	2.9	18	3.1	17	2.9	15	2.9	16	3.0
40-50.....	16	2.9	17	2.8	18	3.1	16	2.8	18	3.0
50-60.....	15	2.9	18	3.1	17	3.0	17	2.9	18	3.1
60-70.....	16	2.9	16	2.7	16	3.1	18	2.8	20	3.6
70-80.....	15	2.8	18	3.0	18	3.0	17	2.7	19	2.8
80-90.....	16	3.0	18	3.0	17	2.8	18	3.1	17	2.7

\* Average weight of plants in 30 untreated checks = 3.5 gm.

TABLE 8

RESULTS OF LEACHING THALLIUM SULFATE IN STOCKTON ADOBE CLAY WITH  
DIFFERENT DEPTHS OF WATER, AS SHOWN BY GROWTH OF INDICATOR PLANTS\*Tl<sub>2</sub>SO<sub>4</sub>, 100 p.p.m. in moistening solution, cultures harvested March 6, 1933

Depth	Leached with 5 cm H <sub>2</sub> O		Leached with 10 cm H <sub>2</sub> O		Leached with 15 cm H <sub>2</sub> O		Leached with 20 cm H <sub>2</sub> O		Leached with 25 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-10.....	5	0.1	5	0.1	7	0.2	7	0.1	7	0.1
10-20.....	13	1.7	13	1.7	13	2.1	13	1.7	12	1.7
20-30.....	13	2.0	13	2.1	14	2.1	13	2.1	13	1.9
30-40.....	13	2.0	13	1.9	14	2.1	14	2.2	13	1.8
40-50.....	13	1.7	14	2.1	13	1.9	14	2.3	14	2.1
50-60.....	12	2.0	13	2.0	14	2.2	14	2.0	14	2.2
60-70.....	14	2.2	14	2.1	14	2.0	14	2.2	14	2.3
70-80.....	14	2.2	13	2.0	13	2.1	14	2.2	14	2.1
80-90.....	14	2.2	13	2.2	13	2.0	13	2.1	14	2.3

Tl<sub>2</sub>SO<sub>4</sub>, 200 p.p.m. in moistening solution, cultures harvested March 20, 1933

Depth	Leached with 37.5 cm H <sub>2</sub> O		Leached with 50 cm H <sub>2</sub> O		Leached with 75 cm H <sub>2</sub> O		Leached with 125 cm H <sub>2</sub> O		Leached with 200 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-10.....	2	0.1	2	0.1	2	0.1	2	0.1	2	0.1
10-20.....	18	2.0	15	1.7	15	2.1	15	1.9	18	1.7
20-30.....	18	2.1	16	1.8	17	1.7	15	1.9	15	1.9
30-40.....	15	1.6	17	2.1	18	1.9	17	2.0	16	1.9
40-50.....	17	2.0	17	1.7	16	1.9	18	1.8	16	1.9
50-60.....	17	1.9	18	1.9	17	1.8	17	2.2	16	1.8
60-70.....	18	2.0	17	1.7	17	1.9	17	1.8	16	2.1
70-80.....	17	2.0	17	2.1	17	2.1	16	1.9	17	2.1
80-90.....	18	2.1	16	1.8	16	1.9	15	1.7	16	1.7

Check tubes moistened with water, cultures harvested March 20, 1933

Depth	Leached with 37.5 cm H <sub>2</sub> O		Leached with 50 cm H <sub>2</sub> O		Leached with 75 cm H <sub>2</sub> O		Leached with 125 cm H <sub>2</sub> O		Leached with 200 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-10.....	15	1.7	15	1.7	15	1.6	15	1.9	15	2.0
10-20.....	15	1.6	15	1.7	15	1.7	15	2.0	15	1.9
20-30.....	15	1.8	15	1.8	15	1.9	15	1.8	15	1.9
30-40.....	15	1.8	15	1.8	15	1.9	15	2.1	15	1.9
40-50.....	15	1.8	15	1.8	16	1.9	15	1.7	16	2.1
50-60.....	15	2.0	15	1.9	16	1.9	15	1.8	15	2.2
60-70.....	15	1.9	15	1.8	15	1.8	15	1.9	15	1.9
70-80.....	15	1.9	15	1.8	15	1.8	15	1.9	16	2.1
80-90.....	15	1.9	15	1.9	15	1.8	15	1.8	15	1.9

\* Average weight of plants in 30 untreated checks = 1.9 gm.

TABLE 9

## RESULTS OF LEACHING THALLIUM SULFATE IN COLUMBIA FINE SANDY LOAM WITH DIFFERENT DEPTHS OF WATER, AS SHOWN BY GROWTH OF INDICATOR PLANTS\*

Tl<sub>2</sub>SO<sub>4</sub>, 100 p.p.m. in moistening solution, cultures harvested March 23, 1933

Depth	Leached with 5 cm H <sub>2</sub> O		Leached with 10 cm H <sub>2</sub> O		Leached with 15 cm H <sub>2</sub> O		Leached with 20 cm H <sub>2</sub> O		Leached with 25 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-10.....	5	0.1	7	0.2	10	0.3	18	0.8	18	1.5
10-20.....	20	3.3	21	3.2	21	3.4	23	3.2	22	3.0
20-30.....	19	3.2	20	3.1	23	3.4	20	2.8	22	3.4
30-40.....	19	3.1	23	3.6	22	3.4	22	3.1	23	3.8
40-50.....	18	3.0	21	3.2	20	2.9	20	3.1	23	2.9
50-60.....	20	3.8	20	3.3	20	3.0	20	2.9	22	3.1
60-70.....	28	3.7	19	3.4	19	2.8	20	2.7	21	2.8
70-80.....	23	3.8	23	3.8	21	3.5	20	2.8	23	3.2
80-90.....	21	4.3	21	3.7	23	3.9	20	2.8	21	2.7

Tl<sub>2</sub>SO<sub>4</sub>, 200 p.p.m. in moistening solution, cultures harvested April 4, 1933

Depth	Leached with 37.5 cm H <sub>2</sub> O		Leached with 50 cm H <sub>2</sub> O		Leached with 75 cm H <sub>2</sub> O		Leached with 125 cm H <sub>2</sub> O		Leached with 200 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-10.....	10	0.3	10	0.7	7	0.6	6	0.5	6	0.3
10-20.....	15	2.3	18	2.3	18	2.4	18	2.2	18	2.3
20-30.....	16	2.5	18	2.4	18	2.7	18	2.2	18	2.7
30-40.....	18	2.2	18	2.5	19	2.3	18	2.8	18	2.3
40-50.....	18	2.5	20	2.7	18	2.6	18	2.2	17	2.3
50-60.....	19	2.4	18	2.5	18	2.7	18	2.4	15	2.7
60-70.....	19	2.4	18	2.5	19	2.8	18	2.6	18	2.9
70-80.....	18	2.5	18	2.7	18	2.5	19	2.6	18	2.7
80-90.....	18	2.4	20	2.6	20	2.7	18	2.7	17	2.4

Check tubes moistened with water, cultures harvested April 4, 1933

Depth	Leached with 37.5 cm H <sub>2</sub> O		Leached with 50 cm H <sub>2</sub> O		Leached with 75 cm H <sub>2</sub> O		Leached with 125 cm H <sub>2</sub> O		Leached with 200 cm H <sub>2</sub> O	
	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
cm	cm	gm	cm	gm	cm	gm	cm	gm	cm	gm
0-10.....	18	2.3	17	2.1	18	2.3	18	2.4	15	2.2
10-20.....	19	2.4	18	2.2	18	2.2	17	2.2	15	2.0
20-30.....	18	2.3	18	2.3	18	2.4	18	2.3	16	2.6
30-40.....	18	2.5	18	2.3	19	2.3	18	2.3	18	2.5
40-50.....	18	2.7	18	2.3	18	2.3	18	2.6	16	2.1
50-60.....	18	2.4	19	2.5	18	2.4	19	2.5	18	2.5
60-70.....	18	2.2	19	2.4	19	2.8	18	2.2	18	2.5
70-80.....	18	2.3	18	2.3	19	2.2	20	2.6	18	2.4
80-90.....	18	2.3	19	2.5	20	2.5	19	2.4	18	2.6

\* Average weight of plants in 10 untreated checks = 5.5 grams for March 23, 1933, cultures; average of those in 30 untreated checks = 3.1 grams for April 4, 1933, cultures.

stimulated growth found in the lower fractions, with little or no leaching, does not occur. Likewise with the greater amounts of water, there is some indication that when the moistening solution contains only 100 p.p.m. of  $Tl_2SO_4$  the toxicity in the top 10 cm becomes somewhat lessened. When the moistening solution carries 200 p.p.m. of  $Tl_2SO_4$ , up to 200 cm of water has no effect upon the toxicity in this top fraction. There is no significant reduction in growth in any of the lower fractions. If leached, the chemical is carried downward in a subtoxic concentration.

With Fresno sandy loam the results are very much the same. Leaching with volumes equivalent to as much as 200 cm of water has no discernible effect upon the location or toxicity of the thallium sulfate within the limits of this experiment. Especially in the less fertile soils, this toxicant is evidently firmly fixed and resists leaching strongly. There is a slight indication that in the Yolo and Columbia soils, where the toxic concentration is higher, when moistening was done with solutions containing 100 p.p.m. of  $Tl_2SO_4$  there was some movement of chemical, and growth was increased. This evidence, however, is hardly conclusive.

*Toxicity of Bait.*—The next experiments were designed to show the effect of thallium-treated grain upon the growth of adjacent plants. Two types of bait were used: potted barley, which had received the normal treatment<sup>8</sup> with thallium sulfate in preparation for field use, and whole barley similarly treated. Kanota oats were used as the indicator plants. Cans containing 500 grams each of dry Yolo clay loam were moistened and planted with the oats. Then the kernels of treated grain were placed in the soil in the same manner as the oats, the distances between the oats and the poisoned grain being varied. Table 10 summarizes the data on these tests, including checks grown at the same time. The whole treated barley germinated and some of the plants grew (table 10). Since the potted barley was heated in the hulling process, the embryos were killed and the kernels did not germinate when planted.

There were planted 470 treated barley seeds in all these tests, of which 246 grew, a survival of practically 50 per cent. These 246 plants weighed 129.9 grams, the average fresh weight per plant being 0.53 gram, 47 per cent of that of the untreated check barley.

In the experiments on the effects of the thallium-treated grain on oat plants, the variation in number of plants per can renders the average weight per plant of little value; but the average total fresh weight per can of oats grown is a fair basis for comparison. These data show that with the 0.25-cm spacing the growth is about 30 per cent less than that of the check plants of untreated oats; with the wider spacing no significant

<sup>8</sup> This treated grain carried 1 per cent  $Tl_2SO_4$  by weight.

differences can be detected. Evidently the sterilizing effect of thallium-coated grain broadcast on the land as bait will be strictly localized and, if the bait is properly scattered, little or no reduction in the natural growth of plants should occur, even if the grain remained on the land through the winter following the application.

TABLE 10

## EFFECTS OF THALLIUM-TREATED GRAIN UPON ADJACENT PLANTS IN YOLO CLAY LOAM

Spacing of plants	Description of plants	Total number of plants	Average number of plants per can	Total fresh weight	Average fresh weight per can	Average fresh weight per plant
Whole barley tests						
0.25	Treated whole barley.....	38	7.6	16.4	3.28	0.43
	Oats, indicator plants.....	37	7.4	23.5	4.70	0.63
	Barley and oats—5 cans.....	75	15.0	39.9	7.98	0.53
0.50	Treated whole barley.....	59	11.8	18.7	3.74	0.32
	Oats, indicator plants.....	56	11.2	39.9	7.98	0.71
	Barley and oats—5 cans.....	115	23.0	58.6	11.72	0.51
1.00	Treated whole barley.....	35	7.0	14.0	2.80	0.40
	Oats, indicator plants.....	59	11.8	45.6	9.12	0.77
	Barley and oats—5 cans.....	94	18.8	59.6	11.02	0.63
2.00	Treated whole barley.....	33	3.3	16.8	1.68	0.51
	Oats, indicator plants.....	77	7.7	91.8	9.18	1.19
	Barley and oats—10 cans.....	110	11.0	108.8	10.86	0.99
Potted barley tests*						
0.25	Oats, total 5 cans.....	50	10.0	41.9	8.38	0.84
0.50	Oats, total 5 cans.....	58	11.6	46.0	9.20	0.79
1.00	Oats, total 5 cans.....	79	15.8	56.2	11.24	0.71
2.00	Oats, total 10 cans.....	76	7.6	94.9	9.49	1.25
Check plants						
	Treated whole barley.....	81	8.1	64.0	6.40	0.79
	Untreated whole barley.....	100	10.0	113.2	11.32	1.13
	Untreated oats.....	100	10.0	117.6	11.76	1.18

\* Growth of indicator oats. Potted barley, being heated in the hulling process, does not germinate.

Another series of tests was made in the greenhouse to find the effect of thallium-coated grain upon growing oats, the bait being applied 10 days after the oats were planted. Twenty cans of Yolo clay loam were moistened and planted on February 7, 1933. The seeds germinated on February 11, and the seedlings were growing rapidly by February 12. On February 17 eight cans received thallium-coated grain (potted-barley

bait) in varying dosages, eight cans received equivalent dosages of thallium sulfate, applied in solution, and four cans remained as checks. The data on these series are given in table 11. The cultures were watered every 2 or 3 days, and the chlorosis characteristic of thallium injury soon appeared. The plants that received the larger doses of thallium sulfate continued to show injury in the cultures, but those receiving less of the chemical showed some signs of recovery toward the end of the test. Judg-

TABLE 11

## EFFECT OF THALLIUM SULFATE FROM BAIT AND IN SOLUTION ON GROWING PLANTS

Tl <sub>2</sub> SO <sub>4</sub> *			Bait treatment				Solution treatment, growth of plants†	
Per culture	Per acre	P.p.m. of air-dry soil	Potted barley*		Growth of plants†		Height	Weight
			Per culture	Per acre	Height	Weight		
gm	lbs.	p.p.m.	gm	lbs.	cm	gm	cm	gm
0.0	0.0	0.0	0.0	0	25	10.7	24	10.0
0.0	0.0	0.0	0.0	0	25	10.9	25	10.4
0.005	8.3	10.0	0.5	830	25	11.0	25	11.3
0.010	16.6	20.0	1.0	1,660	25	10.7	25	11.2
0.025	41.5	50.0	2.5	4,150	25	10.9	24	11.0
0.050	83.0	100.0	5.0	8,300	25	10.0	25	9.2
0.100	166.0	200.0	10.0	16,600	23	8.7	23	4.9
0.150	249.0	300.0	15.0	24,900	23	9.1	20	4.5
0.200	332.0	400.0	20.0	33,200	23	7.9	20	4.6
0.300	498.0	600.0	30.0	49,800	21	5.9	15	1.4

\* In the bait treatment, the thallium sulfate was applied by means of thallium-coated grain; the amount of grain applied to give the dosage is reported in the fourth column.

† Plants per can=10.

ing from these figures, very large dosages of poisoned barley would be required to affect the existing growth of plants in the field. There was a significant difference between the effects of the thallium from the two different methods of application. Apparently the chemical is absorbed by the potted barley and held so that it will not wash off. In these tests the bait lay on top of the soil and was flooded with each irrigation. Probably the chemical that did wash off was quickly fixed in the soil, above the zone of active roots.

*Field-Plot Tests.*—One further experiment was made with thallium-coated grain on square-foot plots in the field. In an enclosure in the corner of a pasture two areas covering approximately 49 square feet each were laid out. After the plots had been treated, the whole was covered with a cage of  $\frac{1}{4}$ -inch mesh galvanized hardware cloth. The treatments were made on February 10, 1933, and the areas were harvested on May 4. Table 12 gives the dosages and weights of harvested plants on these plots.

Two sets of plots were laid out, each on a checkerboard pattern, and all intervening areas were harvested as checks.

The only plots in this test showing significant reductions in yield are Nos. 23 and 48. These received 28.35 grams or 1 ounce each of poisoned grain, scattered evenly over the square-foot area. The cover of grass and range plants was noticeably thinner on these areas. The grain on plots

TABLE 12

## THE EFFECT OF THALLIUM-SULFATE-TREATED GRAIN UPON GROWING PASTURE PLANTS

Plot No.	Dosage per sq. ft.	Fresh weight of crop	Plot No.	Dosage per sq. ft.	Fresh weight of crop
1.....	0.22	153.35	26.....	0.22	76.30
2.....	check	102.35	27.....	check	78.00
3.....	0.44	129.30	28.....	0.44	105.20
4.....	check	121.80	29.....	check	135.15
5.....	0.89	137.70	30.....	0.89	105.00
6.....	check	163.10	31.....	check	101.35
7.....	check	121.80	32.....	check	103.25
8.....	check	151.60	33.....	check	115.45
9.....	check	111.80	34.....	check	114.85
10.....	check	115.10	35.....	check	167.75
11.....	1.77	130.35	36.....	1.77	124.85
12.....	check	85.15	37.....	check	96.00
13.....	3.54	125.00	38.....	3.54	159.80
14.....	check	123.55	39.....	check	127.85
15.....	7.09	111.65	40.....	7.09	150.75
16.....	check	120.45	41.....	check	137.60
17.....	check	129.65	42.....	check	130.90
18.....	check	154.30	43.....	check	158.65
19.....	check	163.10	44.....	check	115.65
20.....	check	147.15	45.....	check	136.70
21.....	14.17	104.85	46.....	14.17	114.90
22.....	check	108.10	47.....	check	95.75
23.....	28.35	85.30	48.....	28.35	87.60
24.....	check	155.10	49.....	check	164.55
25.....	56.70	112.45	50.....	56.70	145.10

25 and 50 was piled in the center of each plot; but the area actually covered was so small that, although bare of vegetation, it had little effect on the yield of the total plot. No. 25 is somewhat reduced in comparison with Nos. 20 and 24, the two adjacent check plots. No. 50 shows no significant reduction. After these plots were laid out, 2.91 inches of rain fell; and the thallium chlorosis could be observed on the plants of the more heavily treated plots while they were young. As the season advanced they seemed to recover; and at harvest time little permanent injury was found, except as noted above. The following year, when these plots were harvested again no significant differences in yield were found on any of them.

## DISCUSSION

The physiological effect of thallium upon plants has not been studied extensively. The differential effect upon the growth rate of shoot and coleoptile of oats is shown in table 3. Since the shoot is formed by cell division in the embryo, whereas the coleoptile develops mainly by enlargement of previously formed cells, meristematic regions may respond characteristically to this element. Chlorosis of older tissues seems to be a constant symptom of thallium poisoning but may be entirely secondary.

Some excellent work has been done at Charles University in Prague by Prat and his colleagues (5) on the absorption of thallium salts from water and from nutrient solutions by plants. Using broad beans and corn, these workers found that practically all the chemical was absorbed within 72 hours from a  $\text{TiNO}_3$  solution  $1 \times 10^{-4}$  molecular in concentration. The plants died in 2 to 4 days. The same amount of thallium nitrate in a nutrient solution (Shive R<sub>s</sub>C<sub>2</sub>) had little effect on the plants. Although they absorbed the nutrient salts, the thallium remained in the solution unchanged in amount for 5 to 10 days, and only 10 per cent to 40 per cent was absorbed in 13 days. Whereas plants readily absorb thallium from pure water solution, but little was taken up from nutrient solutions or from balanced solutions containing  $\text{CaCl}_2$ .

These workers (5) also found a definite effect of thallium upon meristematic cells. These cells take on a mature appearance, and division becomes abnormal and ceases after 48 hours. The illustrations given by them show a pronounced stunting of the roots of plants affected by thallium; large necrotic areas appear on the primary root, and many secondary roots are killed. These effects are much less evident on the plants from the nutrient solutions. Apparently little thallium should be absorbed from soils, especially from those favorable for plant growth.

The writer ashed the tops of several plants that were chlorotic from the presence of thallium in the soil. The ash, moistened with a few drops of concentrated HCl, was heated to dryness and extracted with  $\frac{N}{10}$  HCl.

A sample of the supernatant liquid was sent to Heyrovsky for analysis by means of the Polarograph. Heyrovsky<sup>a</sup> replied concerning the sample: "I could not ascertain any thallium in it." This statement checks with the results of the workers at Prague. Apparently the chlorosis may be a secondary response to the effect of thallium upon the roots. If this element is present in the tops of affected plants, it occurs in such minute amounts that the sensitive Polarograph method cannot detect it. The

<sup>a</sup> Personal correspondence from Professor J. Heyrovsky, June 5, 1933.

roots of these plants were small and unhealthy. Often the plants could be pulled out of the soil, most of the roots breaking off or the xylem pulling out, leaving the cortical tissue behind.

Evidently thallium is very toxic to plants that are growing in water or in poorly balanced solutions. As table 2 indicates, the toxicity of this element varies in different soils, being less toxic in those which are most fertile. Probably lack of fertility reflects a condition in the soil solution that favors absorption of the poison much as does distilled water. Whether this condition is caused by a deficiency in certain mineral nutrients is hard to say without further study; but the assumption seems reasonably well justified, at least in the soils under consideration.

The workers at Prague (5) found appreciable injury to roots of plants in a nutrient solution (Shive R<sub>5</sub>C<sub>2</sub> conc. 0.88 Atm.) containing  $1 \times 10^{-4}$  molecular TlNO<sub>3</sub>. In distilled water containing a like amount of thallium, the plants soon died. This concentration of TlNO<sub>3</sub> corresponds to about 27 p.p.m. in the solution.

In Yolo clay loam (table 2) about 50 per cent growth took place at 60 p.p.m. in the soil, and complete sterility occurred at 240 p.p.m. The corresponding concentrations in the less fertile soil are roughly 46 p.p.m. and 131 p.p.m. The concentrations in the soil solution at field capacity would be three times as great in Yolo clay loam and up to six times as great in lighter soils. Apparently the fixation of thallium compounds in soils renders them less available to plants than they are in solutions. This factor, in addition to the antagonistic action in the balanced solution, makes the critical concentrations in soils fairly high. For complete sterility, apparently, the thallium concentration in the soil must reach 100 p.p.m. or more on a basis of Tl<sub>2</sub>SO<sub>4</sub>. For fertile soils it would be even somewhat higher.

McCool (4) found much higher toxicities in his experiments. Though his method of mixing the chemical in the soil is questionable, probably the more important factors causing these differences were the soils and plant species used. Soils from the humid eastern United States probably compare more nearly with the Fresno and Stockton soils in fertility than with the recent alluvial Yolo and Columbia series. Toxicities would undoubtedly run high in the former soils. On the average, furthermore, the cultivated varieties of plants used by McCool were probably more susceptible than the oats used in the experiments here reported. Most range plants in California would probably be even more tolerant of thallium sulfate in the soil.

Two vital factors are involved in the problem of soil sterility as related to the control of rodents by thallium-treated grain. The first is the

quantity of thallium being placed on the soil per unit area; the second is the final disposition of this poison. To render a soil sterile against annual weeds, one must provide a minimum toxic concentration of the chemical in at least the top inch. An acre-inch of soil weighs roughly 300,000 pounds, and 30 pounds of  $Tl_2SO_4$  would be required to render it sterile. For a 50 per cent reduction in growth, 15 pounds would be needed. Perennial plants would be little affected by even larger doses than this. Considering the fixing power of soils for thallium compounds, even greater amounts of the chemical would be necessary for complete sterilization.

From the high saturation capacity indicated by the data in table 5, an acre-inch of soil could hold up to one hundred times the amount of thallium sulfate required to render it sterile. This fact is important in relation to the distribution of the chemical in rodent control. The usual practice in distributing the bait is to spread one spoonful<sup>7</sup> containing about 20 grams of poisoned grain over an area of 3 to 6 square feet. A bait contains approximately 400 kernels. If each of these was able to sterilize 1 square centimeter (table 10), then seven to fourteen applications would be required to cover the original 3 to 6 square feet, and over 100,000 baits to cover an acre. This would be equivalent to roughly 5,000 pounds of grain bearing 50 pounds of  $Tl_2SO_4$ , and a lethal concentration of the chemical would be provided in the top 1.2 cm of soil if evenly distributed. The actual depth of penetration would probably be much less than this, and many seeds should be able to germinate and grow from below this level. Table 10 also shows that there would be no effect during the first year.

These calculations have been based on the sterilizing capacity of baits lying on the surface of the soil. If the baits were eaten by rodents, the chemical would be distributed, by death of the squirrels, more or less at random, through the top several feet of soil; and immensely greater amounts of thallium would obviously be required to have any appreciable effect. Only animals dying on the surface would leave the thallium in a position to affect the top soil. Such an occasion is rare.

When the problem is viewed from the standpoint of field practice, the disparity between the figures given above and the actual amounts used in rodent control is striking. In an initial campaign with thallium-treated bait, average dosage may run up to a pound of grain per acre or more. Because of the effectiveness of this poison, however, the dosage may be rapidly reduced. In one California county the average dosage had decreased to  $\frac{1}{35}$  of a pound of bait per acre in five years. Since this

<sup>7</sup>A standardized spoon of definite size is used for this purpose.

bait carried only 1 per cent  $\text{Tl}_2\text{SO}_4$  by weight, evidently the chemical reaching the soil is negligible as compared with that required for sterilization.

Though the results of these studies are of little value in the actual field of soil sterilization and are mostly negative in relation to the rodent-control problem, one point seems noteworthy. If sterilization of soil by thallium-treated grain should ever occur, it would result from the accumulation of untaken baits. This grain would also be a source of danger to other animals and would represent a waste of material. This poison, therefore, should be handled by competent and experienced men so that the majority of baits will be placed where they will be taken. If this precaution is observed, soil sterilization is not a factor in the control of ground squirrels with thallium-treated grain.

It is regrettable that such warnings as those of Brooks (1, p. 106) and McCool (4, p. 295) should be issued without some preliminary study of the actual field practice involved.

#### SUMMARY

Experiments indicate that thallium sulfate is very toxic in soils. Thirty pounds will sterilize an acre-inch of average soil. Toxicity decreases, however, with time and cropping.

Thallium toxicity varies with soil type, a range of three times or more having been shown in the soils studied.

The toxicity of thallium is greater in soils low in fertility. It cannot be correlated with the soil type nor with water-holding capacity.

Thallium toxicity is evidenced by retarded shoot growth, a nearly normal development of the coleoptile, chlorosis of leaves, stunting of older plants, and early death where the concentrations are high.

Thallium sulfate was strongly fixed in four soils. The saturation capacity of Yolo clay loam for this chemical was about 10,000 p.p.m. on a dry-weight basis.

Leaching with as much as 200 cm of distilled water had practically no effect upon the thallium toxicity in these soils.

Thallium-treated barley, as commonly used for squirrel bait, had little or no effect upon germination or growth of oats planted in the same can and spaced within 0.5 cm of the grains. Growth was reduced when the spacing was 0.25 cm. Thallium-treated whole barley gave a 50 per cent germination, and the fresh weight of the seedlings at 30 days was 47 per cent of that of the checks.

Oat seedlings were unaffected by the application of treated barley to the soil, followed by irrigation, except where the dose was excessive.

Thallium-treated barley also had little effect upon growing plants in a pasture area. The heaviest application, equivalent to over 2,500 pounds of grain to the acre, reduced the growth less than 50 per cent.

Workers at Charles University, Prague (5), have shown that plants do not readily absorb thallium salts from balanced nutrient solutions. Ashed plants from the test here reported failed to give a thallium test by the sensitive Polarograph method of Heyrovsky.

Thallium sulfate in concentrations of 100 p.p.m. or more on a dry-weight basis should be completely toxic in most soils. The concentration at saturation would be around a hundred times this value.

About 30 pounds of thallium sulfate uniformly distributed would be required to sterilize an acre-inch of soil. Under natural conditions of application it would probably be tied up in a much shallower layer of soil. At least 5,000 pounds of squirrel bait, carrying 1 per cent  $Tl_2SO_4$ , uniformly distributed, would be necessary to sterilize an acre completely.

If the baits are taken by squirrels, the thallium is distributed at random in localized regions in the top several feet of soil. Under these conditions the dosage mentioned above would give no sterilization except where an animal might die on the surface.

In actual field practice, dosage seldom exceeds 1 pound of thallium-treated grain per acre, bearing 0.01 pound of  $Tl_2SO_4$ . Dosage rapidly decreases as the rodents are brought under control.

The differences between these rates of dosage and those mentioned above show that little need be feared from the sterilization of soils by thallium-treated squirrel bait.

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TOXICITY OF ARSENIC, BORAX, CHLORATE,  
AND THEIR COMBINATIONS IN THREE  
CALIFORNIA SOILS

A. S. CRAFTS AND C. W. CLEARY



# TOXICITY OF ARSENIC, BORAX, CHLORATE, AND THEIR COMBINATIONS IN THREE CALIFORNIA SOILS<sup>1</sup>

A. S. CRAFTS<sup>2</sup> AND C. W. CLEARY<sup>3</sup>

## INTRODUCTION

IN WEED CONTROL it is often desirable to apply two or more chemicals at the same time. Where, for example, both annuals and deep-rooted perennials occur, arsenic and chlorate combined may be used for complete sterilization. In such cases one must know the reciprocal effects of these reagents in order to use them with any assurance of success. This paper describes experiments designed to show the toxicity of three common herbicides used two and three at a time in three California soils.

## TOXICITY STUDIES

Tests on the toxicity of sodium arsenite and sodium chlorate (2)\* and sodium borate (3) used separately, in these soils have been published and the technique has been described. Briefly, it consists in growing indicator plants, Kanota oats in this case, in soil cultures in No. 2 cans. The chemicals being studied are added to the air-dry soil, dissolved in sufficient water to bring the soil to its field capacity. The cultures are then seeded and grown for 30 days, at which time the height and fresh weight of the indicator plants are recorded.

In the preliminary toxicity tests (2, 3) concentration series were used covering the complete range from 0 to 100 per cent toxic and beyond, so that cultures were included that showed no plant growth even after several croppings. In the present experiments two arbitrary growth levels were selected : the 50 per cent level at which growth was reduced to approximately one-half that of the untreated checks, and the 10 per cent level at which growth was correspondingly reduced to a low value. The concentrations used to produce growth at these two levels were derived from the original toxicity curves, the data from the first runs with the three chemicals being computed in terms of percentage of checks. Table 1 gives the values expressed as parts per million in terms of the air-dry soil. For the borax they have been converted to the hydrous form that contains 47 per cent water by weight.

<sup>1</sup> Received for publication May 8, 1936.

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\* Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

In these experiments the chemicals were applied in solution as the sodium salts—that is, sodium acid arsenite,  $\text{NaH}_5(\text{AsO}_3)_2$ ; sodium tetraborate,  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ; and sodium chlorate,  $\text{NaClO}_3$ . These are the forms in which the chemicals are presented on the market and in which, consequently, they are most conveniently purchased for weed control. Other forms of the same toxic elements may readily be converted to this basis if their composition is known.

The letters *A*, *B*, and *C* are used for convenience in expressing results. *A* designates arsenic, expressed as  $\text{As}_2\text{O}_3$  and applied as sodium acid arsenite; *B* hydrous sodium tetraborate; and *C* sodium chlorate.

TABLE 1

RATES OF APPLICATION OF ARSENIC, BORAX, AND CHLORATE GIVING 50 PER CENT AND 10 PER CENT GROWTH IN THREE CALIFORNIA SOILS

Soil type	Per cent growth	Arsenic ( <i>A</i> ) $\text{As}_2\text{O}_3$	Borax ( <i>B</i> ) $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$	Chlorate ( <i>C</i> ) $\text{NaClO}_3$
		p.p.m. in air-dry soil		
Yolo clay loam.....	50	300	300	100
	10	540	540	540
Fresno sandy loam.....	50	45	130	40
	10	140	240	200
Stockton adobe clay.....	50	100	700	36
	10	200	1,000	120

The plan of the experiments may best be explained in connection with the presentation of data in table 2. In addition to checks receiving only tap water, each soil at the two growth levels received each chemical in four concentrations as shown in the column headed "Rate." A value of 4 in this column represents an application equal to the whole amount required to reduce growth to the level designated. For instance, in Yolo clay loam at the 50 per cent growth level, opposite the rate 4 in the arsenic set, the crop was 16 cm in height and weighed 6.1 grams. This culture received 300 p.p.m. of  $\text{As}_2\text{O}_3$ . In Fresno sandy loam at the 10 per cent level opposite the rate 4 in the chlorate set the crop was 8 cm in height and weighed 0.3 gram. This culture received 200 p.p.m. of  $\text{NaClO}_3$ . The rates, 3, 2, and 1 represent dosages of  $\frac{3}{4}$ ,  $\frac{1}{2}$ , and  $\frac{1}{4}$  of this basic rate respectively. In the first case cited above the dosages were 75 p.p.m., 150 p.p.m., 225 p.p.m., and 300 p.p.m.  $\text{As}_2\text{O}_3$ . Each column of cultures in each chemical set therefore represents a short concentration series consisting of an untreated check and four concentrations, the highest concentration being intended to reduce the growth in the cultures to the particular growth level under consideration.

TABLE 2

## TOXICITY OF ARSENIC, BORAX, CHLORATE, AND THEIR COMBINATIONS, AS SHOWN BY GROWTH OF INDICATOR PLANTS

Application		Yolo clay loam				Fresno sandy loam				Stockton adobe clay			
		50 per cent		10 per cent		50 per cent		10 per cent		50 per cent		10 per cent	
Chemicals	Rate*	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.	Ht.	Wt.
Arsenic	0	15	10.5	14	9.0	28	5.4	26	5.0	15	1.5	15	1.8
	1	17	9.3	15	8.0	28	5.0	24	3.2	14	1.0	12	0.8
	2	16	7.6	13	4.8	28	4.5	15	1.2	10	0.7	10	0.6
	3	16	6.9	13	2.7	27	4.0	10	0.6	9	0.5	9	0.4
	4	16	6.1	6	0.6	25	3.3	8	0.4	9	0.5	8	0.3
Borax	0	16	9.3	14	10.0	28	5.4	27	5.2	15	1.4	14	1.6
	1	16	9.9	15	9.7	30	4.9	29	4.0	16	1.3	14	1.1
	2	17	8.0	17	7.6	30	4.3	30	3.3	15	1.1	15	1.2
	3	17	8.1	13	4.0	32	4.2	24	2.0	17	1.2	11	0.6
	4	17	6.4	6	0.9	29	3.0	17	0.7	15	0.9	4	0.1
Chlorate	0	17	10.7	15	9.6	28	5.6	27	4.9	16	1.7	15	1.7
	1	10	6.8	11	2.5	31	4.7	23	2.0	15	1.3	7	0.4
	2	17	5.7	8	1.0	28	3.2	19	1.1	9	0.6	5	0.2
	3	16	5.0	5	0.3	28	3.1	9	0.5	8	0.4	5	0.2
	4	15	4.0	5	0.2	26	2.5	8	0.3	6	0.2	4	0.1
A+B	4+0	16	6.1	6	0.6	25	3.3	8	0.4	9	0.5	8	0.3
	3+1	17	7.0	9	2.0	25	3.1	9	0.4	12	0.8	8	0.2
	2+2	17	7.2	14	3.7	28	3.3	9	0.4	13	0.7	8	0.2
	1+3	18	7.5	12	3.2	30	3.2	13	0.5	14	0.8	8	0.3
	0+4	17	6.4	6	0.9	29	3.0	17	0.7	15	0.9	4	0.1
A+C	4+0	16	6.1	6	0.6	25	3.3	8	0.4	9	0.5	8	0.3
	3+1	15	5.6	6	0.4	28	2.9	8	0.4	10	0.8	7	0.3
	2+2	16	5.0	5	0.4	27	2.5	9	0.4	10	0.6	5	0.2
	1+3	16	4.5	4	0.3	25	2.6	8	0.4	7	0.3	5	0.2
	0+4	15	4.0	5	0.2	26	2.5	8	0.3	6	0.2	4	0.1
B+C	4+0	17	6.4	6	0.9	29	3.0	17	0.7	15	0.9	4	0.1
	3+1	18	8.0	10	2.0	30	3.7	24	1.5	14	1.0	8	0.5
	2+2	16	7.1	13	2.4	30	4.2	23	1.7	15	1.1	9	0.7
	1+3	17	5.9	14	1.7	27	3.1	20	1.4	13	0.8	5	0.2
	0+4	15	4.0	5	0.2	26	2.5	8	0.3	6	0.2	4	0.1
A+B+C	1+2+1	17	7.3	14	3.1	32	5.2	16	1.1	14	0.8	10	0.5
	1+1+2	18	6.4	13	2.2	30	4.0	19	1.0	12	0.9	7	0.4
	2+1+1	18	6.6	14	2.7	29	3.9	11	0.6	11	0.8	9	0.4
	4 4 4 + + +	18	6.6	14	2.7	30	4.1	16	0.9	12	0.9	10	0.5
	3 3 3 + + +	18	6.6	15	10.1	28	5.5	27	5.1	16	1.5	16	1.7
Checks†	0	17	10.4	15	10.1	28	5.5	27	5.1	16	1.5	16	1.7

\* "Rate" is expressed in quarters of the amount of chemical necessary to produce the desired growth levels; see explanation on page 402.

† Average of 17 replicates.

In the combination sets the three chemicals were used two at a time. They were combined in the proportions designated in the "Rate" column. These values refer strictly to the concentrations of the designated chemicals: in the *B + C* set, 2 + 2 means that in Yolo

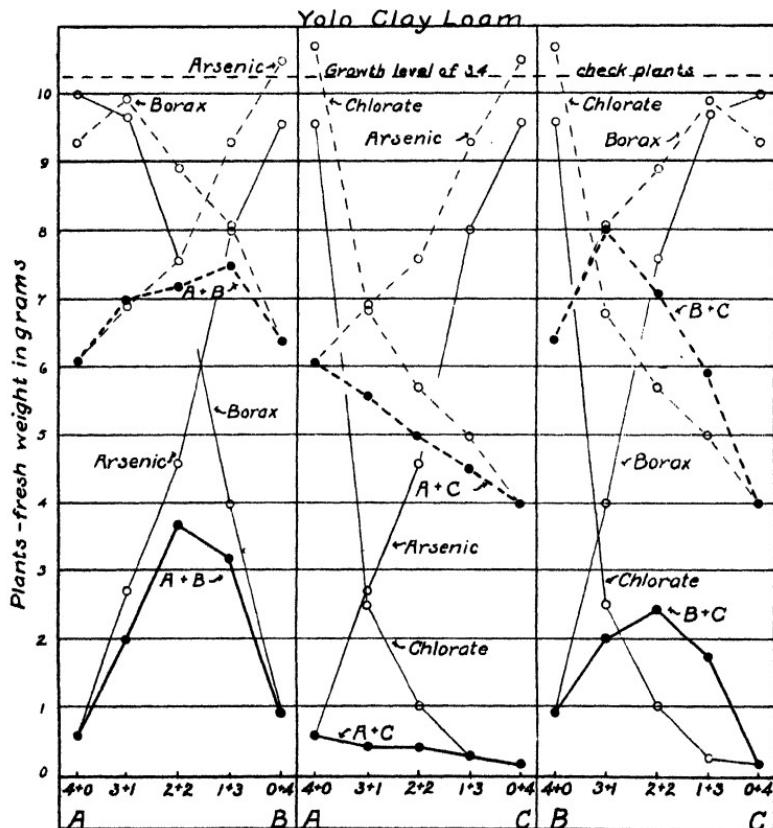


Fig. 1.—Toxicity of arsenic, borax, chlorate, and their combinations used two at a time in Yolo clay loam. Solid lines in lower part of the figure indicate the 10 per cent growth level, and dotted lines in the upper part, the 50 per cent growth level.

clay loam, for example,  $\frac{1}{4}$  of 300 p.p.m. of borax (table 1) and  $\frac{1}{4}$  of 100 p.p.m. of chlorate (table 1) were combined at the 50 per cent level. The dosages were therefore 150 p.p.m. of borax and 50 p.p.m. of chlorate respectively, not equal dosages of each. The same rule applied where all three chemicals were used in combination.

In each soil there were 34 different treatments, run in triplicate, and 8 checks for each growth level. The values given in table 2, except the

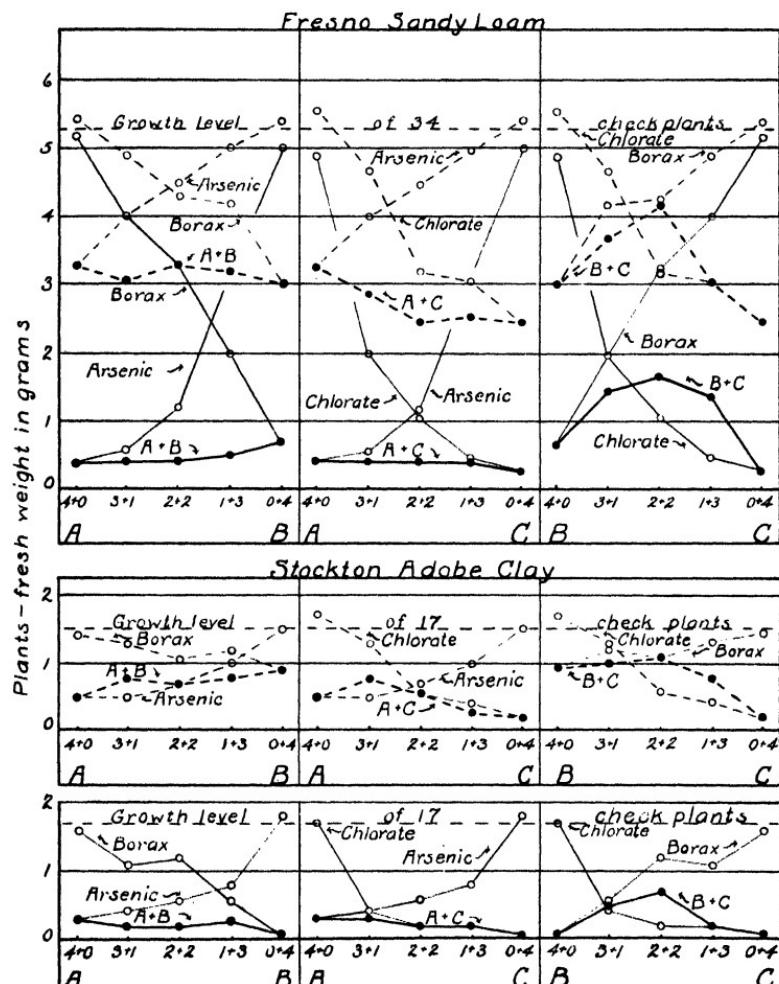


Fig. 2.—Toxicity of arsenic, borax, chlorate, and their combinations used two at a time in Fresno sandy loam and Stockton adobe clay. Solid lines in lower part of the figure indicate the 10 per cent growth level, and the dotted lines in the upper part the 50 per cent growth level.

checks at the end, represent the average of the three replicates. The values for the checks at the end of the table include the 8 unattached checks in addition to the 9 included in the single chemical sets shown above.

To facilitate the interpretation of the data figures 1, 2, and 3 have been prepared. The first two show the toxicity curves for the single chemicals and their combinations when used two at a time; figure 3 shows the relations of the chemicals used singly and used three at a time.

When herbicides are used in combination there are three possible types of response. The effects might be strictly additive. That is, at a given growth level the result of chemical treatment should be the same whether the dosage is all applied as one chemical or another, or as combinations of the two, of such amounts as to total the same in all cases. Considering, for example, the curves in figure 2 for arsenic and chlorate in Fresno sandy loam at the 10 per cent level, it made little difference whether the application was made of 4 increments of arsenic, 3 of arsenic plus 1 of chlorate, 2 of each, 1 of arsenic plus 3 of chlorate, or 4 of chlorate. In all five cases the results were essentially the same.

The second possibility is that the two chemicals might be antagonistic in their action, and the resultant reaction upon the plant might be less from mixtures than from either alone. This is the case with the chlorate-borax mixtures in all three soils.

The third possibility is that the total effect upon plants of the combination treatment might be greater than that expected on the basis of the sum of the individual effects of the two taken alone. This type of response was not observed with any of the mixtures used in these experiments.

There are three types of toxicity curves in figures 1 and 2: straight-line curves such as that for arsenic at the 50 per cent level in the Fresno sandy loam; curves that are concave as viewed from above, such as the arsenic and chlorate curves at the 10 per cent level in Fresno sandy loam; and curves that are convex when viewed from above, as the borax curves in the Yolo clay loam.

The straight-line curve indicates that all increments of a single chemical are of equal value. The concave curves indicate a high toxicity for the first increments and decreasing effectiveness as more are added. Chlorate-toxicity curves are usually of this form if the total dosage approaches the zero growth level. Convex curves indicate low toxicity in the low applications and increasing effectiveness as a lethal dosage is approached.

Evidently the combination curves in figures 1 and 2 are influenced in

practically every experiment by the form of toxicity curves of the two component chemicals. In the Yolo clay loam there is antagonism between arsenic and borax at both growth levels, with toxicity much lower than would be expected on the high-borax side. In the arsenic-chlorate combinations there is in a sense no antagonism, since the curves connecting the two extremes are practically straight lines at both growth levels; but there is considerable difference in the effectiveness of the different increments of each chemical, the first of chlorate being particularly toxic in both cases.

In the borax-chlorate experiment there is distinct antagonism, the combination curve crossing that for chlorate alone, a fact indicating that the borax detracted from the effectiveness at the high-chlorate—low-borax end. This response, though related to the convex form of the borax curve, is more pronounced than would be expected from the results with those two chemicals alone.

In the Fresno sandy loam the borax is much more toxic (table 1) and in combination with arsenic shows no marked reduction in toxicity. Arsenic and chlorate also show straight-line curves for the combinations in this soil. Borax and chlorate again show antagonism with the combination curves crossing the chlorate curves. This same behavior is shown in Stockton adobe clay, where borax toxicity is extremely low. Since it occurs in all three soils it is probably related to the chemicals and relatively independent of the soil type. The arsenic-borax and arsenic-chlorate curves in the Stockton soil are essentially straight lines, a fact indicating that these combinations have little or no antagonistic action in both this and the Fresno soil.

Evidently the combinations used three at a time (fig. 3) are all less toxic than equivalent dosages of the single chemicals; and in some cases, as for example the 10 per cent growth level in Yolo clay loam, toxicities are markedly low. Since this type of mixture has only theoretical interest and is of little practical value, it will not be considered further.

One might judge from the foregoing discussion that these studies have yielded little useful information, since no mixtures of outstanding effectiveness have shown up. Such, however, is not the case. In the first place, it should be pointed out that in the future the principal use of these chemicals in combination will be in soil sterilization, a process that will gain in popularity as agricultural production comes under a higher degree of control. And very probably they will be applied dry wherever possible, for this is the least expensive method.

Among the dry chemicals used in soil sterilization, arsenic comes first in toxicity and retention in available form in the top soil (1, 2). In the

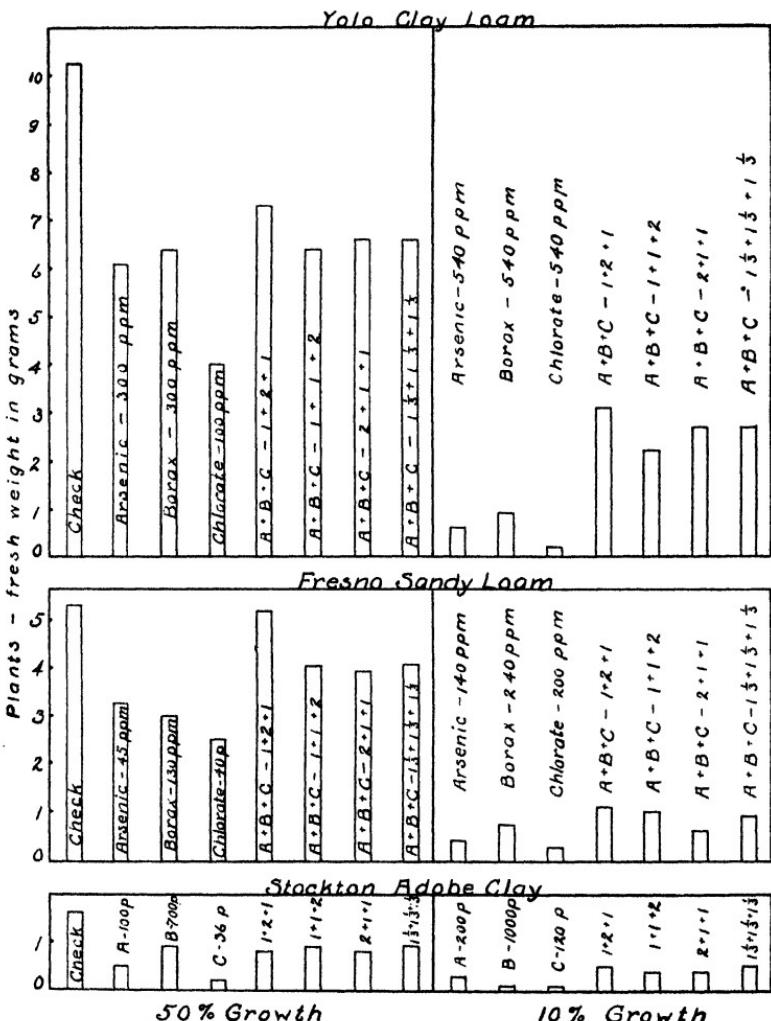


Fig. 3.—Toxicity of arsenic, borax, chlorate, and their combinations used three at a time as shown by growth of indicator plants.

form of the trioxide, however, it is only slightly soluble, and at least one season is required, under California conditions, for enough to become available to give effective sterilization. There is need for an agent that may be combined with it to kill vegetation during the year of application.

Borax, though extremely toxic to many plants when first applied to the soil, soon loses in effectiveness with time and leaching (3). It is more firmly retained in the top soil than chlorate and is nonpoisonous to live-stock (3). Chlorate, being very soluble, is not held against the force of moving water in most soils. It is valuable, in consequence, for treating deep-rooted perennials and will kill annuals during the year of application in California, especially if applied in the late winter or spring.

Considering now the mixtures: arsenic and borax, both being surface sterilants, would have little or no use in combination. Though borax is nonpoisonous and might be substituted for arsenic, it is not satisfactory as a quick killer for annual weeds. In Yolo soils these chemicals are antagonistic.

Chlorate, being soluble and a quick killer, will combine with dry white arsenic to form a very desirable mixture for soil sterilization. Since they are not antagonistic in their action, these reagents may be used in any proportions; and where sufficient chlorate is incorporated, deep-rooted perennials may be eliminated in the process.

On the basis of our data the borax-chlorate combination might seem to hold little promise, since the action of these chemicals was antagonistic in all three soils. Several advantages, however, may be gained by using this mixture. Both chemicals are nonpoisonous as applied in this method; the fire hazard in the use of the chlorate would be practically eliminated; the effects would be considerably more durable than in the use of chlorate alone; and the cost could be materially reduced.

Experiments indicate that colemanite may be used as a substitute for borax (3). This fact would place this chemical on a cost basis of about one-tenth that of chlorate, and a very effective mixture of the two could be marketed at a nominal cost. The principal problem is to prescribe the proportions for mixing and the dosages to be used on different soils and under various climatic conditions.

The shape of the toxicity curves of borax and chlorate indicates that the most effective increments of chlorate are the first, whereas the higher applications of borax have the most pronounced effect. The combination curves indicate, furthermore, a high antagonism where little borax was combined with much chlorate. Evidently, therefore, in combining these two chemicals one should use a minimum effective dosage of chlorate and should add borax as the need is indicated by the soil type and local

conditions. From table 1 little chlorate is required to reduce growth 50 per cent. In two soils less borax than chlorate is needed to lower it from 50 per cent to 10 per cent, and in the Stockton soil less than four times as much borax as chlorate is required. Considering the 1 to 10 differential in price, it seems logical to use chlorate through its range of maximum effectiveness and then to add borax enough to finish the destruction. With the added residual effect of the borax, this combination should prove economical. It is excelled only by arsenic and chlorate, a mixture which has limited use because of its poisonous nature.

### DISCUSSION

The foregoing considerations indicate the use that may be made of greenhouse technique in building a body of information upon which to base an interpretation of field results. The limitations of the method should also be pointed out. As several years of experience have shown, the toxic concentrations of the various chemicals studied are not absolute but depend somewhat upon growth conditions of the indicator plants. Borax and chlorate are evidently absorbed and translocated in plants and tend to accumulate in the leaves. The toxicity of these reagents is affected, therefore, by conditions determining rates of absorption and water loss. In addition, toxicity is rapidly reduced during the initial stages of any experiment of this type; and although this loss can be shown only by comparing successive crops, it is going on from the time the first crop is planted. Since toxicity loss varies in rate with different chemicals and under different environmental conditions, comparative studies with two or more chemicals are limited in accuracy. For such reasons the concentrations required to reduce growth to certain fixed levels may not always be the same, and with the best of judgment the worker may miss the desired points. This was the case in the Yolo soil, where both the arsenic and borax concentrations used failed to bring growth to the 50 per cent level, while the chlorate concentration took it below this line. Similar discrepancies can be observed in the other two soils, which indicate the general nature of the disturbing factors. Though these discrepancies interfere somewhat with the results, the general responses are so apparent that their value is little depreciated. The convenience and adaptability of the method far outweigh its drawbacks as is indicated by the conclusions drawn.

These same factors that limit the accuracy of the greenhouse method affect the results of field applications. Chemical treatments for soil sterilization are subject not only to such obvious factors as rainfall, temperature, soil type, and species susceptibility, but to all those complex

relations that determine the crop-producing power of soils and their ability to fix and retain solutes against the force of moving water. For the present the best that can be done in determining the behavior and effects of herbicides in soils is to study, by the empirical methods described, the growth of plants in the treated soils. Such studies are providing abundant information, sufficiently accurate to aid materially in the design and ultimate interpretation of field-plot studies.

### SUMMARY

In the greenhouse experiments described, sodium arsenite, hydrous sodium tetraborate, and sodium chlorate are used singly and in combination to reduce growth of indicator plants.

Concentrations of these three chemicals required to reduce growth to the 50 per cent level and to the 10 per cent level in Yolo clay loam, Fresno sandy loam, and Stockton adobe clay were derived from previously published data.

In the present experiments, check series were set up in each soil, the individual chemicals being applied in increments of  $\frac{1}{4}$ ,  $\frac{2}{4}$ ,  $\frac{3}{4}$ , and  $\frac{4}{4}$  of that required to reduce growth to the specified level. Each such set, constituting a short concentration series, was used as a basis for comparing the combination treatments.

In experiments combining the chemicals two at a time, they were applied in proportions of  $4 + 0$ ,  $3 + 1$ ,  $2 + 2$ ,  $1 + 3$ , and  $0 + 4$ . The chemicals used three at a time were combined in the proportions of  $1 + 2 + 1$ ,  $1 + 1 + 2$ ,  $2 + 1 + 1$ , and  $\frac{1}{3} + \frac{1}{3} + \frac{1}{3}$ .

Arsenic and borax showed antagonistic reaction in Yolo clay loam at both the 50 per cent and the 10 per cent growth levels.

Arsenic and borax toxicities were additive in Fresno sandy loam and Stockton adobe clay.

Arsenic and chlorate toxicities were additive in all three soils at both growth levels.

Borax and chlorate showed antagonistic reactions in all three soils at both growth levels.

The combination of the chemicals used three at a time are of only theoretical interest and provide no practical information.

In the practical application of these chemicals the arsenic-borax combination would find little use.

Sodium chlorate and white arsenic applied dry, form a very useful mixture for soil sterilization. As shown by the greenhouse experiments, there is no indication of loss by antagonism in their reactions.

In the use of borax and sodium chlorate in combination for soil sterili-

zation the antagonism in their action can be reduced to a minimum by using the lowest effective dosage of chlorate and adding enough borax to complete the destruction of the vegetation.

The borax-chlorate combination for soil sterilization has the advantage of being practically nonpoisonous; and the use of the borax, besides reducing the fire hazard of the chlorate to a low level, provides a residual effect that lowers the probability of reinfestation by seedlings.

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**ONTOGENY AND STRUCTURE OF  
COLLENCHYMA AND OF VASCULAR TISSUES  
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## DEVELOPMENT OF THE FLOWER AND MACRO- GAMETOPHYTE OF ALLIUM CEPA<sup>1</sup>

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IN CENTRAL CALIFORNIA mother bulbs of the onion (*Allium cepa* L.) used for seed production are usually set in the field during late November and December. Subsequently, a number of leaves are formed at each of the several growing points before the inflorescence axis is differentiated (fig. 1, A). The method of leaf development has been adequately described by Hoffman.<sup>14</sup> Briefly, the leaves are two-ranked, the blade of each new leaf arising at an angle of 180° from that of the next older. That side of the apical meristem opposite the preceding blade is the first to differentiate; and as this region develops an upward growth of tissue soon completely encircles the growing point of the stem, differentiating the new leaf.

In Maryland, Jones and Boswell<sup>15</sup> found that the primordium of the inflorescence axis differentiated in March when mature bulbs were planted in the field in October. In California, bulbs planted in December had floral axes differentiated in February.<sup>16</sup> The first stages in the development of the primordium of the leaf and that of the inflorescence axis appear to be very similar. In the latter the single involucral bract is first evident at a point opposite the youngest leaf blade. At the first appearance of the bract, one cannot tell whether the new primordium is that of a leaf or of a bract. Very soon, however, the central axis begins to

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<sup>4</sup> Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

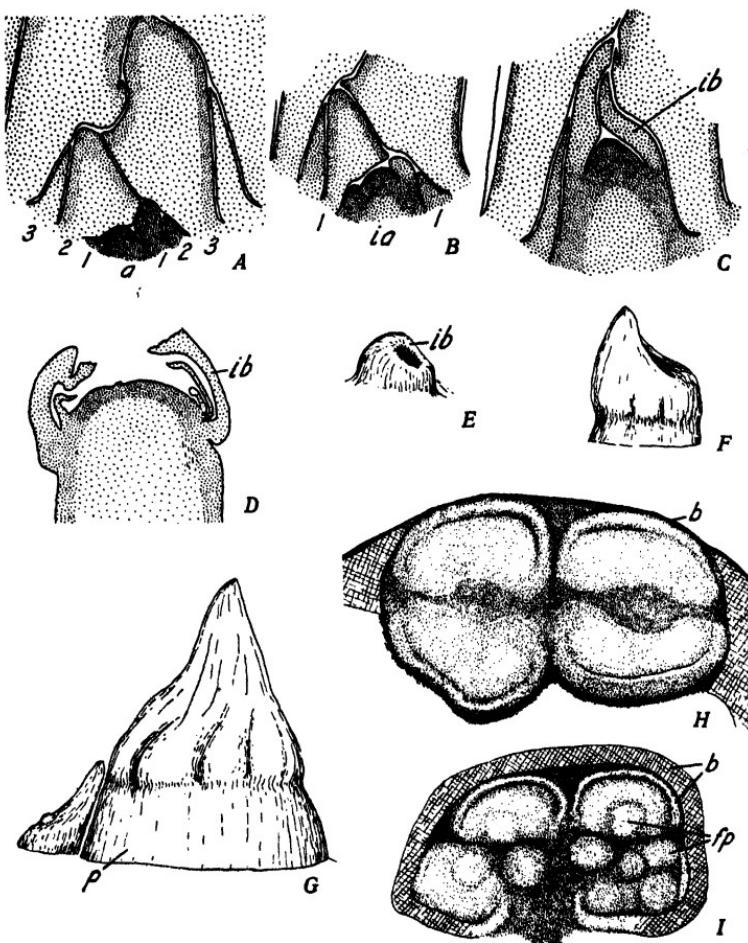


Fig. 1.—A, Longitudinal section through the growing point of onion bulb: 1, 1; 2, 2; 3, 3, are opposite portions of the same leaf that encloses the central axis *a*. B, Primordium of the inflorescence axis. Involucral bract just arising; 1, 1a are opposite sides of the same leaf encircling the inflorescence axis, *ia*. C, Elongating peduncle of the inflorescence axis. The involucral bract, *ib*, completely covers the central region. D, Apex of inflorescence axis somewhat flattened; additional bracts arising within the involucre. E, Inflorescence axis is in about the same stage of development as in B, showing the unilateral development of the involucre. F, G, Developing flower stalks. At the base of the peduncle in G another shoot has differentiated. H, Within the young bracts are slightly raised kidney-shaped areas from which most of the flowers develop; *b*, bracts. (x about 32.) I, The primordia of the first flowers are being differentiated: *b*, bracts; *fp*, flower primordia. The bract covering four kidney-shaped areas has been cut away. (x about 32.)

elongate to form the peduncle or scape of the inflorescence (fig. 1, *B* and *C*), and the involucral bract covers the meristematic region from which the flowers develop (fig. 1, *C*, *F*, and *G*). Within the involucre and over the broad surface of the stem tip, numerous membranous bracts develop, which cover the cluster of young flowers in their first stages (plate 2, *A*). In other words, during their early development, the flowers are protected by the involucre as well as by another series of bracts within. Figure 1, *E* shows how the single involucral bract in a very early stage completely encircles the growing point.

### FLOWER DEVELOPMENT

The individual florets are preceded by a varying number of slightly elevated kidney-shaped meristematic regions (fig. 1, *H*) over the surface of the stem apex. It is mainly from these kidney-shaped areas that the flower primordia are differentiated. The plan or order of differentiation seems fairly definite but the exact sequence of development was not determined.

In the onion there are three members in each of the five whorls of floral organs; these whorls are outer perianth, inner perianth, outer stamens, inner stamens, and carpels. The flower primordium develops at first as a slight projection (fig. 1, *I*), then becomes globose. Later it is slightly elongated with a convex summit and is circular in cross section (fig. 2, *A* and *B*). By marginal growth just below the summit the primordia become somewhat flattened and when viewed from above distinctly triangular (plate 2, *B* and plate 3, *A*). The outer perianth whorl and the outer stamen whorl are the first to be formed. At each angle of the triangle there is differentiated a primordium of an outer perianth segment, and in its axil a primordium of one of the stamens of the outer whorl. Occasionally the first lobe of the perianth is well developed before the primordium of the stamen which it subtends is evident. Usually, however, these two appear to arise simultaneously from a single growing region, an outer primordium giving rise to the perianth segment and an inner to the stamen. The second perianth segment with its subtended stamen, as a rule, arises counterclockwise to the first (fig. 2, *G*). The remaining apex of the triangle also develops a rounded protuberance (fig. 2, *C*), which in turn develops two rapidly growing regions, the primordia of the third perianth segment and its subtended stamen.

The inner whorls of perianth and stamens are formed after the outer. The first of the inner perianth segments with its subtended anther appears between the oldest and second oldest segments of the outer whorl

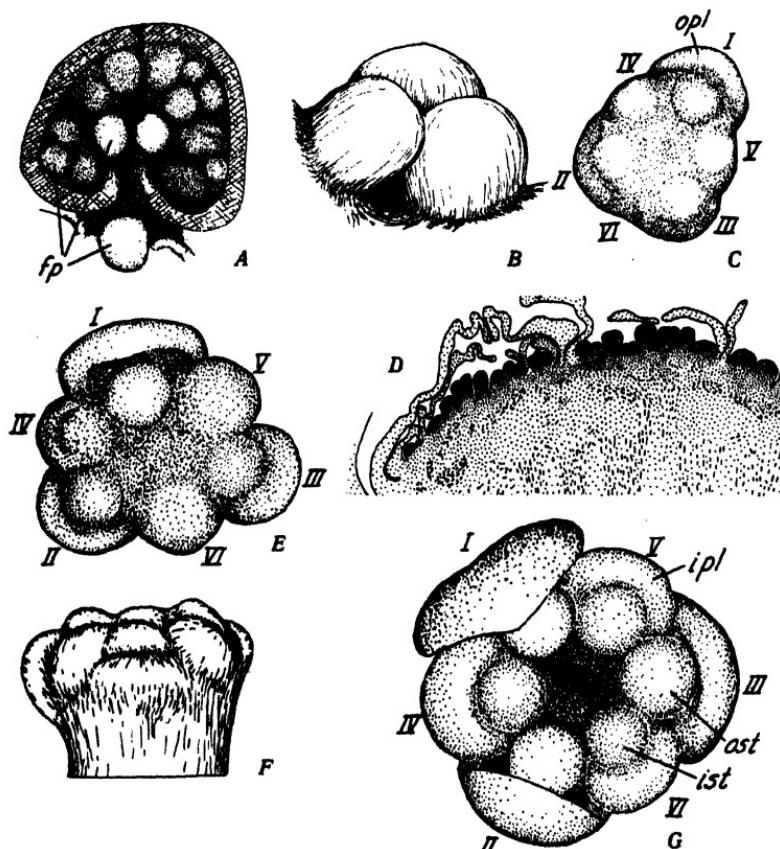


Fig. 2.—*A*, Slightly older stage than figure 1, *I*; *fp*, flower primordia. (× about 32.) *B*, Side view of three flower primordia. (× about 85.) *C*, Top view of a young flower showing counterclockwise differentiation of outer perianth lobes and outer whorl of stamens. The youngest primordium (*vi*) will appear opposite the oldest perianth segment (*I*); *opl*, outer perianth lobe. (× about 85.) *D*, Longitudinal section of a portion of inflorescence at about the time the floral organs of the oldest flowers are beginning to differentiate. *E*, Top view of a young flower showing outer whorls of perianth and stamens differentiated; the inner whorls are just beginning to differentiate. The Roman numerals indicate the sequence of origin of the different segments. (× about 85.) *F*, Side view of a young flower slightly younger than the one shown in *E*. (× about 85.) *G*, Top view of a young flower in which primordia of all perianth segments and stamens have been differentiated: *ipl*, inner perianth lobe, *ast*, outer stamen, *ist*, inner stamen. (× about 85.)

(fig. 2, E). The next members appear between the oldest and youngest segments of the outer whorl. Occasionally this sequence is reversed. As a rule the last segments to arise are opposite the oldest (figs. 2, C; 2, E; and 3, B). Sometimes segments of the inner whorls lying both clockwise and counterclockwise to the oldest segments of the flower appear to arise simultaneously. Even though the inner stamens are the last to arise, they are the first to shed their pollen.

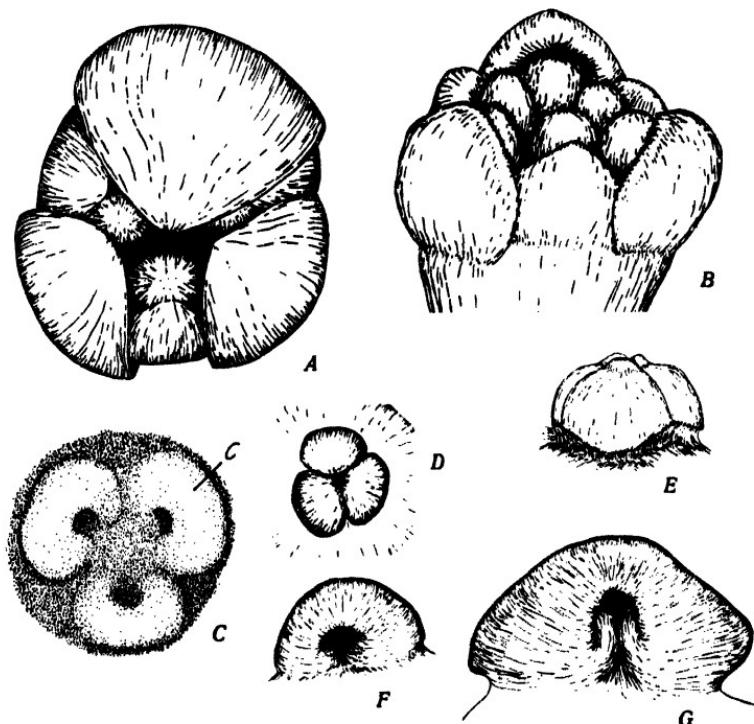


Fig. 3.—A, Top view of a young flower. The outer perianth lobes almost cover the stamens. At about this time the carpels are differentiated. ( $\times$  about 85.) B, Side view of a young flower, slightly younger than A. ( $\times$  about 85.) C, Early differentiation of the three carpels. ( $\times$  about 85.) D, Carpels have grown upward until they nearly meet at the center. ( $\times$  about 32.) E, Carpels with the style just beginning to form. All carpels play a part in the formation of the style. ( $\times$  about 32.) F, Inturned edges of a single carpel, showing origin of the two ovules. ( $\times$  about 85.) G, Slightly older carpel than F. ( $\times$  about 85.)

The carpels are differentiated at about the time the outer perianth segments overarch the stamens (fig. 3, A). Three horseshoe-shaped areas of meristematic tissue begin to project on the flattened surface within the three inner stamens and alternate with them. These areas, growing upward and toward the center (fig. 3, C), soon meet along their inturned

edges (fig. 3, *D*). The style is formed by the apical growth of the three carpels (fig. 3, *E*). When the three carpels begin to elongate to form the style, the primordia of the ovules have already been differentiated on the inner edges of the carpels (fig. 3, *F* and *G*). In figures 4, *D* and 4, *E* a

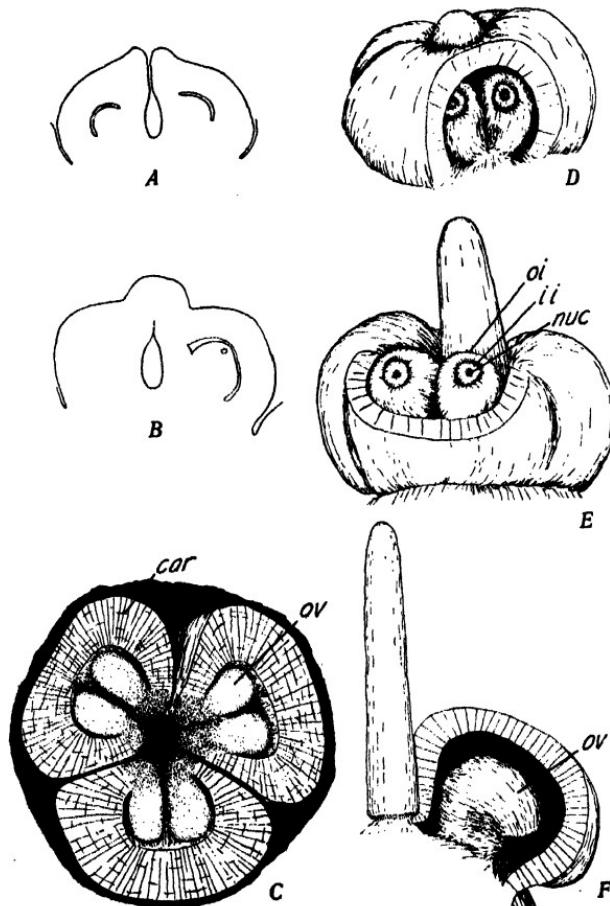


Fig. 4.—*A*, Longitudinal section through two carpels about the same age as in figure 3, *E*. *B*, Stage of development at which the archesporial cell is differentiated. *C*, Upper portion of carpels (*car*) removed, showing young ovules (*ov*). (× about 85.) *D*, *E*, Portion of a carpel removed to show the two ovules, outer (*oi*) and inner (*ii*) integuments, and nucellar (*nuc*) tissue. (× about 32.) *F*, Longitudinal section through one of the locules at time of anthesis.

section of the ovary wall has been removed, exposing the two ovules within the carpel. Figure 4, *F*, the pistil of an open flower, shows the almost complete inversion of the ovule.

### DEVELOPMENT OF THE MACROGAMETOPHYTE

The archesporial cell, which is subepidermal, functions directly as the macrospore mother cell. This cell can be distinguished among the other nucellar cells at a very early stage, even before the integuments begin

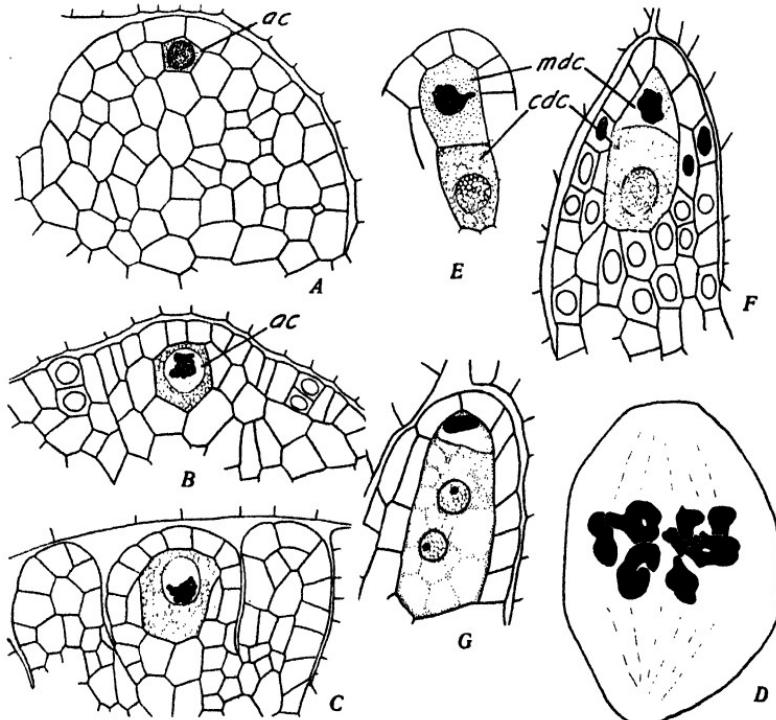


Fig. 5.—*A*, Ovule with differentiating archesporial cell (*ac*) which in this case is the megasporangium. ( $\times$  about 375.) *B*, Mother cell in early prophase. The inner integuments are being initiated by periclinal division in the epidermis. ( $\times$  about 375.) *C*, Enlarging mother cell still in prophase. The integuments have made considerable development. ( $\times$  about 375.) *D*, Heterotypic division of the mother cell. ( $\times$  about 1,250.) *E*, Two daughter cells: the micropylar daughter cell (*mdc*) begins to disintegrate shortly after division; the chalazal daughter cell (*cdc*) forms the embryo sac. *F*, Enlarging of the inner daughter cell to form the embryo sac. ( $\times$  about 375.) *G*, Two-nucleate embryo sac. ( $\times$  about 375.)

to form. When the inner integument first begins to differentiate (fig. 5, *B*), the prophase has already begun. Immediately following the first metaphase (fig. 5, *D*), the micropylar daughter cell begins to disintegrate but persists for some time (figs. 5, *E* and *F*); the other forms the embryo sac. This degeneration of the micropylar daughter cell in *Allium*

*cepa* is characteristic of many species of *Allium* and occurs in other genera as well.

The origin of the embryo sac from the chalazal daughter cell has also been described by Strasburger<sup>(6)</sup> for *Allium fistulosum*; by Weber<sup>(9)</sup> for *A. porrum*, *A. victoriale*, *A. paniculatum*, *A. flavum*, *A. ursinum*, *A. zebdanense*, *A. uniflorum*, *A. rotundum*, and *A. sphaerocephalum*. These have only flowers in the inflorescence. In *A. carinatum*, *A. oleraceum*, *A. scorodoprasum*, *A. moly*, *bulbiferum*, *A. paradoxum*, and *A. sativum*, which have both bulbils and flowers in the inflorescence, Weber<sup>(9)</sup> states that the embryo sac is also formed directly from the chalazal daughter cell; but aberrations from this type are more frequent than in species with only flowers in the inflorescence. Heatley<sup>(10)</sup> found that in *Trillium cernuum* the embryo sac developed from the inner of the two daughter cells. In *Streptopus roseus*, McAllister<sup>(11)</sup> writes that in most cases the eight-nucleate embryo sac was derived from the inner daughter cell. In *Scilla hyacinthoides* var. *caerulea* and *S. campanulata*, McKenney<sup>(12)</sup> reports that the embryo sac develops from the micropylar daughter cell. In *Cypripedium*, Pace<sup>(13)</sup> found that it was derived from the inner daughter cell, but the mature sac had four nuclei instead of eight.

In *Allium cepa* the chalazal daughter cell undergoes successive mitosis to form the eight-nucleate embryo sac. In an embryo sac, from material collected four days after the stigma had first become receptive, the egg was still unfertilized (plate 1, *B*), and the polar nuclei were separate (plate 1, *C*). The small synergid (plate 1, *D*) and egg were of about the same size. The volume of the large synergid (plate 1, *E*) was several times that of the small one; both were richly supplied with food. Even when the stigma is first receptive the contents of the two synergids are much denser than those of the egg (plate 4, *A* and *B*).

In the genus *Allium* the behavior of the synergids is not the same for all species. Weber<sup>(9)</sup> reports that in *A. senescens* and *A. victoriale* the synergids disappear immediately after fertilization; *A. flavum*, *A. paniculatum*, *A. ursinum*, and *A. zebdanense* have slight hypertrophy of the synergids, and these probably provide food until the embryo reaches the two to three-celled stage. Weber states that in *A. uniflorum* and *A. rotundum* one of the synergids acts as a nutritive cell and does not disintegrate until the embryo has developed a large number of cells. Strasburger<sup>(6)</sup> pictures one of the synergids of *A. fistulosum* as much hypertrophied and filled with granular protoplasm. In *A. cepa* the persistence of the synergids was not determined. No doubt the large synergid functions, for a short time at least, as a nutrition organ.

## SUMMARY

The outer perianth whorl and the outer stamen whorl are the first floral organs to differentiate in the onion. These are followed by the inner perianth whorl and inner stamens, and lastly by the carpels.

The outer perianth segments and their subtended anthers usually arise counterclockwise. The sequence of development of the members of the inner whorls of perianth and anthers is usually clockwise. The first segments of the inner whorls usually arise between the oldest and second oldest segments of the outer whorls.

The embryo sac is formed from the chalazal daughter cell.

One of the synergids is distinctly hypertrophied; both appear to be well supplied with reserve food.

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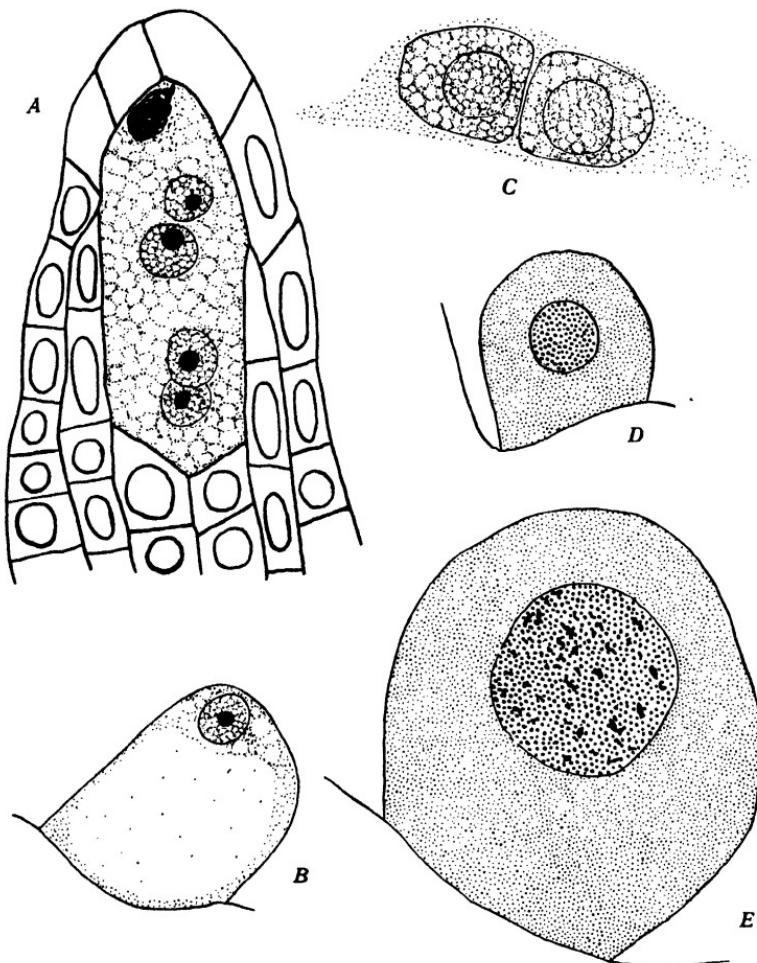


Plate 1. *A*, Four-nucleate embryo sac. ( $\times$  about 375.) *B-E*, Polars, synergids, and egg from the same sac, four days after stigma is first receptive: *B*, egg; *C*, polars; *D*, small synergid; *E*, large synergid. ( $\times$  about 875.)

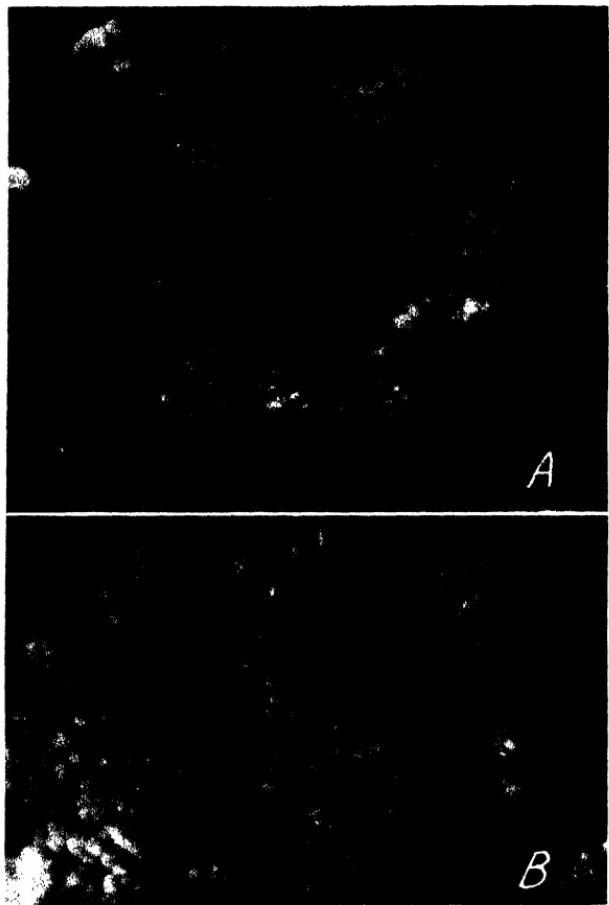


Plate 2. *A*, Photomicrograph of a portion of young inflorescence, involucre removed, young flowers covered with membranous bracts. *B*, Young inflorescence with portion of membranous bracts removed to show the developing flowers.

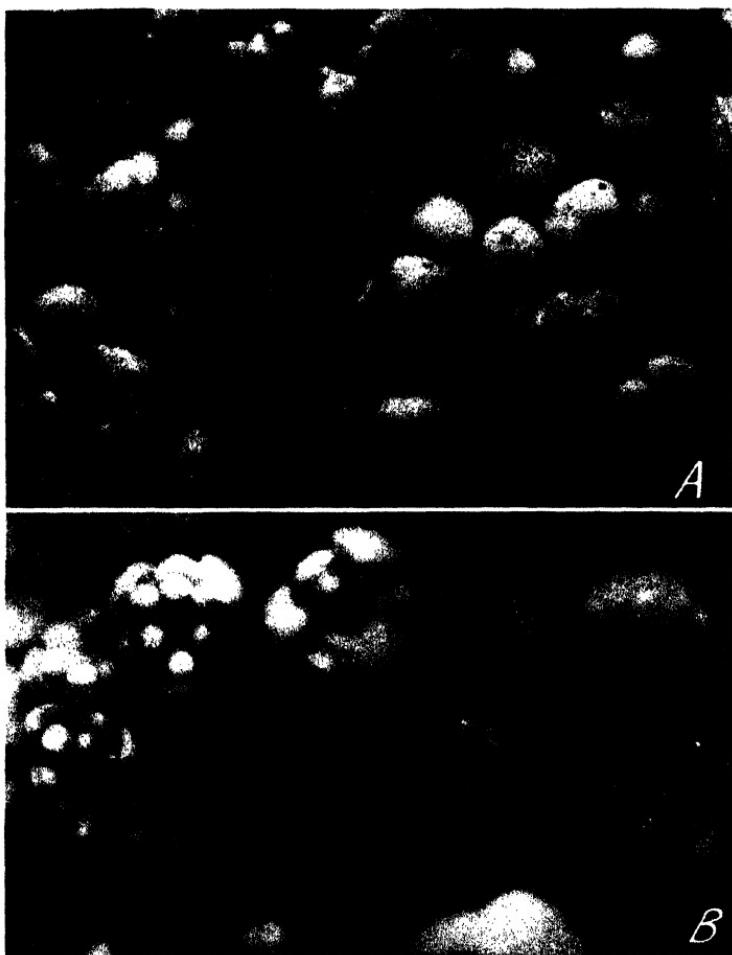


Plate 3. *A*, Flower primordia at the time the floral organs are beginning to differentiate.  
*B*, Perianth segments and stamens have differentiated.

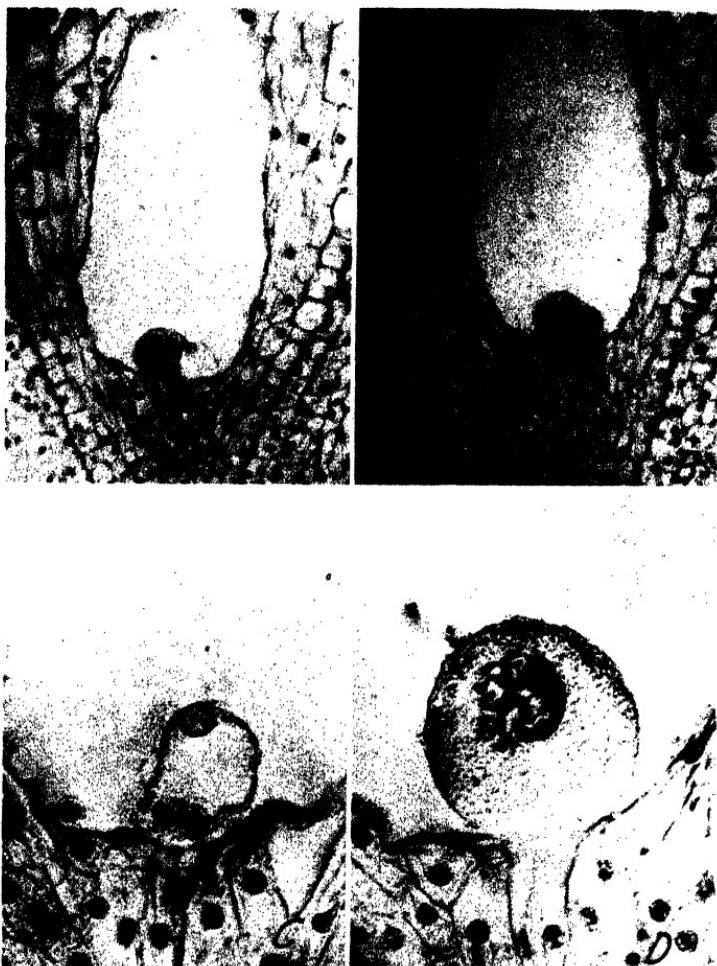


Plate 4. *A, B*, Two sections of the same sac at the time the stigma is first receptive: *A*, large synergid and egg; *B*, two synergids. *C, D*, Two sections of the same sac four days after the stigma was first receptive: *C*, egg; *D*, large synergid.

**ONTOGENY AND STRUCTURE OF  
COLLENCHYMA AND OF VASCULAR TISSUES  
IN CELERY PETIOLES**

**KATHERINE ESAU**



# ONTOGENY AND STRUCTURE OF COLLENCHYMA AND OF VASCULAR TISSUES IN CELERY PETIOLES<sup>1</sup>

KATHERINE ESAU<sup>2</sup>

## INTRODUCTION

EFFORTS TO OBTAIN STRINGLESS varieties of celery (*Apium graveolens* L.) drew attention to the nature of the so-called celery strings. The two structures that constitute these strings, the vascular bundles and the collenchyma strands, differ greatly from each other in their development, their histology, and their physical properties. This paper deals with the ontogeny and structure of the tissues that make up the strings and gives some information regarding their relative strength.

The present work treats of the histological part of the problem in considerable detail and adds to our knowledge of histogenesis and tissue differentiation. It compares the mode of origin of collenchyma with that of the vascular tissue and describes in detail the phloem and its transformation, in the final stages, into the collenchymatous bundle cap.

## MATERIAL AND METHODS

In preparing the material for histological study, the procedure was as follows. Pieces of petioles were killed and fixed for 48 hours in Karpechenko's chrom-acetic-formalin solution (Rawlins, 1933,<sup>3</sup> p. 13). The fixed material, washed in three changes of pure dehydrated dioxan (Graupner and Weissberger, 1931), was placed in a paraffin oven in a mixture of dioxan and paraffin. The latter was changed four times to expel the dioxan. Three to four days after placement in the oven the material was embedded in paraffin.

Without being soaked in water, the embedded material was cut 10 microns thick on a rotary microtome. Instead of a microtome knife, Gillette razor blades, clamped into a Spencer razor-blade holder, were used. Very little difficulty was experienced in cutting the material, even the collenchyma and xylem of the oldest petioles. The protoplasts, however, commonly shrank throughout the material, though no attempt was made to determine at what stage of the process this shrinkage occurred.

For staining, Heidenhain's haematoxylin was used in most cases,

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<sup>3</sup> See "Literature Cited" at the end of the paper for complete data on citations, which are referred to in the text by author and date of publication.

although the combination safranin-anilin blue was also resorted to. The haematoxylin stain was prepared according to Hance (1933). The anilin blue, used in a weak solution in 90 per cent alcohol, was applied to sections previously stained in safranin. It rapidly stains the cytoplasm and walls of cellulose and gives a good contrast with safranin, which is retained by the lignified walls, the nucleoli, and the slime of sieve tubes.

Preparation of the material for comparing the relative strength of collenchyma and of the vascular bundles is mentioned in connection with the method of measuring this strength.

The material used for the illustrations was taken from three celery plants, each of a different variety. Figure 1, *A* to *C* was drawn from sections through the growing point and the youngest leaves of the Utah variety. Figures 1, *D* to *I*, figures 2-5, and plates 1-8 were taken from the Golden Plume plant. Table 3 gives the origin of the material for figures 7 and 8.

#### GROSS ANATOMY OF PETIOLES

Celery petioles have crescent shapes in transverse sections, with prominent ribs on the abaxial (dorsal) side (plate 1, *A*). A large collenchyma strand is present in each rib under the epidermis (plate 2). No collenchyma strands occur on the adaxial (ventral) side, but here the two to three subepidermal layers of cells are collenchymatously thickened (plate 2). The two lateral points of the crescent are also prominently reinforced by collenchyma.

The main vascular system of the petiole is distributed on its abaxial side and is composed of vascular strands of various sizes (plates 1 and 2). Each collenchyma strand lies on the same radius with one of the larger bundles; the smaller bundles have no collenchyma strands opposing them (plate 2). A row of very small vascular bundles occurs on the adaxial side (plates 1 and 2). The vascular strands are embedded in large-cell parenchyma with intercellular spaces. In pithy plants this parenchyma breaks down in certain regions, leaving prominent internal cavities (plate 2).

Oil ducts are a conspicuous feature of the celery petioles. There is an oil duct subjacent to each collenchyma strand (plates 2; 3; 4, *A*; and 6); numerous others occur in the parenchyma surrounding the vascular bundles (plates 1, *B*; and 3). Oil ducts also differentiate in the metaphloem (plate 5).

The collenchyma strands are more uniform in size and are fewer than the vascular bundles. The largest bundles exceed the collenchyma strands in diameter (plate 2).

The large vascular bundles are collateral; and the xylem occurs on the adaxial, the phloem on the abaxial side of the bundles (plate 2). The xylem, roughly triangular in cross-sectional outline, is covered dorsally and laterally by a crescent-shaped structure that includes the phloem and the bundle cap (plates 2 and 5). In mature bundles the xylem is

TABLE 1

## CERTAIN GROSS MORPHOLOGICAL CHARACTERISTICS OF CELERY PETIOLES

Variety	Leaf No.	Length of petiole, cm	Area of median cross section, $\text{cm}^2$	Collenchyma		Vascular bundle		
				Number of strands	Av. size of strand, $\text{mm}^2$	Number of bundles	Av. size of bundle, $\text{mm}^2$	Av. size of bundle cap, $\text{mm}^2$
Golden Plume.....	11	7	3.1	..	..	..	..	..
	12	9	3.1	22	0.273	33	0.648	0.396
	14	10	3.0	..	..	..	..	..
	15	10	3.2	..	..	..	..	..
	16	11	..	16	0.514	23	0.905	0.525
	17	17	3.3	..	..	..	..	..
	18	12	3.1	..	..	..	..	..
	21	14	3.2	..	..	..	..	..
	24	15	..	13	0.663	17	0.862	0.500
	26	15	..	13	0.503	13	0.873	0.506
Tall Golden Self-blanching.....	10	14	0.9	..	..	..	..	..
	12	15	..	15	0.135	17	0.364	0.225
	13	20	0.9	..	..	..	..	..
	15	20	1.0	..	..	..	..	..
	16	24	..	15	0.163	16	0.460	0.236
	17	22	0.9	..	..	..	..	..
	23	15	..	12	0.193	13	0.474	0.276
	24	18	..	12	0.258	14	0.636	0.332
	25	18	1.2	..	..	..	..	..
	26	16	..	12	0.242	13	0.605	0.336
Utah.....	9	9	1.9	15	0.176	16	0.556	0.352
	14	20	1.9	14	0.248	17	0.718	0.438
	15	24	1.9	..	..	..	..	..
	16	21	2.4	16	0.258	17	0.744	0.479
	17	24	1.9	..	..	..	..	..
	18	23	1.9	..	..	..	..	..
	22	21	2.2	..	..	..	..	..
	23	19	1.8	15	0.257	15	0.809	0.500

separated by cambium from the functioning phloem (plate 5). The latter is a small-celled tissue, which, on the outside, merges with the tissue of the bundle cap.

Some of the small bundles, especially those on the adaxial side, consist of phloem only. Solitary xylem vessels without the accompanying phloem also occur. If xylem and phloem are both present in the adaxial bundles, their relative position is reversed as compared with that in the

abaxial bundles; that is, the xylem lies on the abaxial, the phloem on the adaxial side of the bundle.

The collenchyma and the large vascular bundles occur as continuous parallel strands extending from the base of the petiole to the place where the lamina is inserted. The vascular strands may have a few anastomoses connecting them laterally with each other. Both the collenchyma and the vascular strands have a larger diameter at the base than at the apex. From the base up they decrease rather rapidly in thickness and then remain fairly uniform to the apex of the petiole.

Table 1 shows certain size relations of parts of celery petioles in three different plants. The area of the petiole was measured in its median cross section. The areas of the strands of collenchyma and of the vascular tissues represent averages of basal and apical areas of all strands of a given cross section of the petiole, except of the smallest vascular strands, which had no corresponding collenchyma strand and were not measured. The number of vascular bundles represents the sum of abaxial bundles, including the smallest. The number of collenchyma strands is the sum of abaxial strands, except the collenchyma reinforcing the two marginal ribs.

The areas of the vascular bundles and of the collenchyma strands were drawn from their transverse sections by means of a camera lucida at a magnification of 44 diameters and were then measured with a planimeter.

The number of each leaf indicates its relative distance from the growing point. In counting, a leaf 0.5 cm in length, located near the growing point, was given the number 1, so that the highest number indicates the oldest leaf in each plant.

As table 1 shows, the first leaves on the plant tend to have fewer vascular bundles and fewer collenchyma strands than those formed later. The vascular strands average considerably larger in diameter than the corresponding collenchyma strands.

Plates 1 and 2 show size relations of parts of petioles in three stages of development. These photographs, all of the same magnification, were taken at the base of petioles of leaves 1, 7, and 13 of the Golden Plume plant.

#### HISTOGENESIS OF PETIOLES

A leaf primordium has a very broad base and a narrow apex, which soon becomes lobed and gives rise to the pinnate leaf blade. The base develops into a petiole through elongation, tissue differentiation, and maturation.

Using Schüepp's (1926, p. 4) general classification of meristems, we may distinguish three kinds of meristematic tissue in the primordial

petiole: the protoderm, the procambium, and the ground meristem. In this terminology, procambium is the meristem that gives rise to the primary xylem and phloem and the vascular cambium; the protoderm differentiates into the epidermis; the ground meristem into the pith and cortex.

The three meristems may be distinguished on the basis of size and degree of vacuolation of their cells. The ground-meristem cells are comparatively large, particularly on the adaxial side of the petiole; and they are the first cells to develop conspicuous vacuoles and intercellular spaces. The protoderm has small cells, somewhat more dense than those of the ground meristem. The least vacuolate and the smallest in cross section are the procambium cells, which are also distinguished by being longer than broad. Cell-division figures are more abundant in the protoderm and in the procambium than in the ground meristem.

As seen in transverse section, a petiole in the primordial state is crescent-shaped, like the more mature one. Considering a cross section through the thickest part of the petiole of a leaf approximately 250 microns high, we find, beginning with the adaxial side (fig. 1, A, beginning below), the following layers: (1) the adaxial protoderm, consisting of one layer of cells; (2) several layers of comparatively large cells of the ground meristem (the adaxial meristem of Foster, 1935a); (3) the procambial region with small-cell procambium strands and slightly larger embryonic cells intervening between these strands; (4) two to three layers of abaxial cortical ground meristem; and (5) one layer of abaxial protoderm.

The cells of the protoderm divide only anticlinally. In the ground meristem two types of divisions occur—periclinal and anticlinal. In the procambium, periclinal divisions generally predominate; but anticlinal walls are also formed.

The procambial tissue of a leaf is initiated in the ground meristem (see also Foster, 1935a, p. 116) of the primordium through a rapid succession of periclinal longitudinal divisions. The procambium strands of the larger vascular bundles arise earlier than those of the smaller bundles. The small abaxial bundles and all the adaxial bundles are initiated in those regions of the ground meristem where vacuolation is conspicuous. Foster describes a similar late initiation of procambium in the lateral strands of the cataphylls of *Carya* (1935a, p. 105) and in the cortical bundles of transitional leaf forms of the same plant (1935b, p. 172).

In the Umbelliferae, according to Ambronn (1881), collenchyma differentiates in close connection with the vascular bundles; in fact,

Ambronn states that they both arise from the same procambium strand; but through differentiation of a layer of parenchyma within the procambium strand the latter becomes separated into two parts—the outer, giving rise to collenchyma, and the inner, forming the vascular tissue.

The histogenesis of celery petioles deviates from the scheme given by Ambronn for the Umbelliferae. As was mentioned earlier, approximately three layers of cells separate the procambium strand from the protoderm during the first stages of tissue specialization (fig. 1, *A*). Later very active periclinal divisions occur on the abaxial side of the procambium strand, the resulting cells becoming arranged in radial rows. Such cells are shown in figure 1, *B* between the procambium (*pr*) and the oil duct (*od*). In view of the absence of a clear demarcation between the procambial and the ground-meristem cells on the periphery of the procambium strand, it is difficult to determine accurately whether these cells are derived from the outer procambium cells or from the ground meristem. Through the appearance of anticlinal walls in later stages of development the original radial arrangement of the cells is lost (fig. 1, *E*).

Immediately outside this active region an oil duct differentiates before any xylem or phloem elements are in evidence in the petiole (fig. 1, *B*). The oil ducts arise as schizogenous intercellular spaces, but may be distinguished from ordinary intercellular spaces by their rather large size and the characteristic divisions of cells lining the space.

During the developmental processes just described, important changes are initiated in the layers located between the oil duct and the protoderm. Although the ground-meristem cells of this region early manifest vacuolation and development of intercellular spaces, they undergo sporadic divisions (fig. 1, *A* and *B*). After the appearance of the oil duct, divisions become increasingly numerous (figs. 1, *C* and 5, *A*); and, in consequence, a strand of elongated dense cells, similar to procambial cells, is formed. This strand increases in size and subsequently differentiates into collenchyma.

Through the rapid division of cells on the abaxial side of the procambium strand, and the divisions leading to the formation of collenchyma, the abaxial portion of the petiole lying on the same radius with the procambium strand becomes elevated in the form of a rib. The ground-meristem cells between the ribs adjust themselves to the active abaxial elevation by increase in size and by a few divisions.

Developmental processes occurring on the abaxial side of large bundles do not take place near those small bundles which are not associated with collenchyma strands (fig. 1, *C*, right).

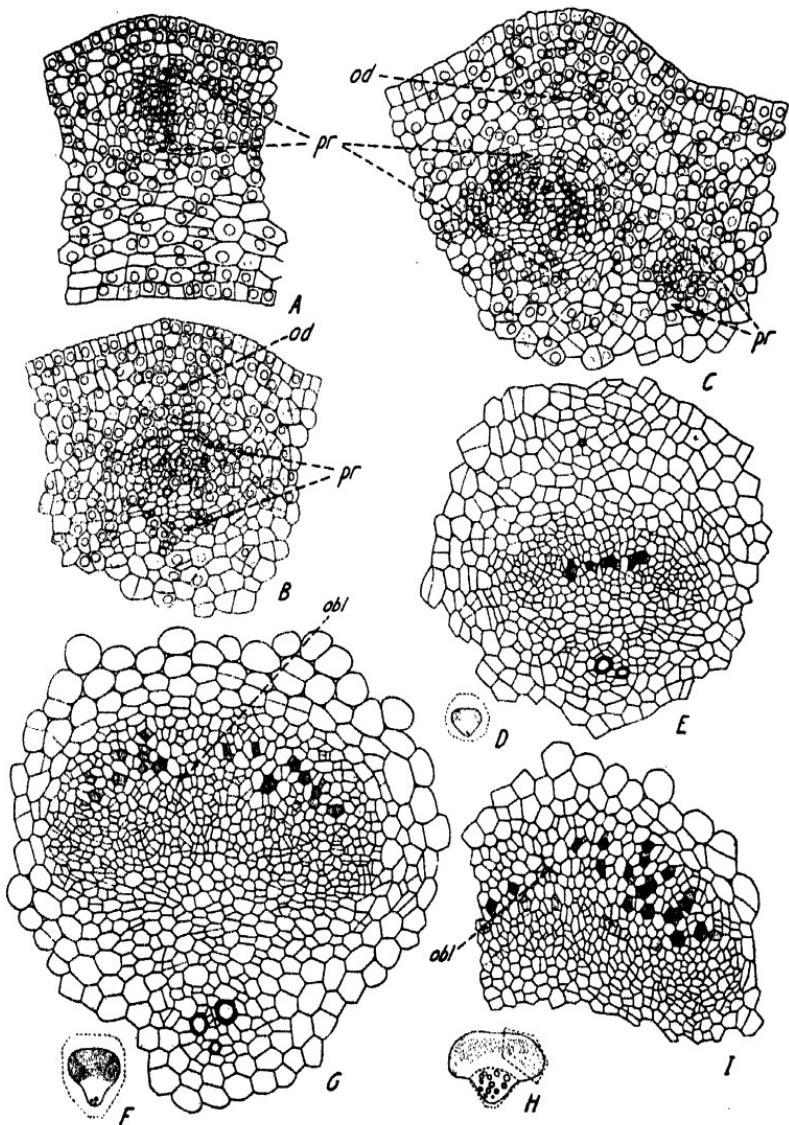


Fig. 1.—A-C, Transverse sections through parts of petioles showing differentiation of procambium strand ( $\times 184$ ); E, G, I, early differentiation of vascular tissues in transverse sections ( $\times 184$ ); D, F, H, diagrams of entire bundles from which drawings E, G, I were made ( $\times 38$ ); obl, obliteration; od, oil duct; pr, procambium.

The description given above shows that, in contrast to the Umbelliferae studied by Ambronn (1881), celery is characterized by the independent origin of collenchyma and vascular bundles within the petioles. The early differentiation of the oil duct facilitates recognition of regions and clearly limits the primordial collenchyma to the subepidermal layers of the ground meristem.

Although Ambronn (1881) considers that in the Umbelliferae the collenchyma and the vascular tissue arise from the same procambium strand, in most other dicotyledons he found these two kinds of tissues to arise independently of each other. None of the plants studied by Wisselingh (1882) offered an example of common origin of collenchyma and vascular tissue. The Umbelliferae, however, were not included in Wisselingh's material.

#### ONTOGENY AND STRUCTURE OF THE VASCULAR BUNDLES

*Procambium and Cambium.*—In the formation of the procambium in petioles, longitudinal divisions in certain regions of the ground meristem occur in rapid succession, forming strands of elongated cells with small transverse diameters. These divisions are periclinal and anticlinal; but as the anticlinal walls tend to be formed perpendicularly to the periclinal ones, the procambial cells show rather regular radial arrangement (fig. 1, A). In the phloem region of the procambium strand this arrangement is soon destroyed by the appearance of the less regularly placed anticlinal walls (fig. 1, C, E, and G). In the xylem region the radial arrangement is lost after the vessels, enlarging, induce divisions and spatial adjustments in the xylem parenchyma (fig. 1, E and G; plates 3 and 4). In the region of the strand where cambium is to differentiate, the radial rows of cells remain in evidence because periclinal divisions predominate here.

In longitudinal sections the procambium cells are longer than broad because the periclinal divisions are not immediately followed by transverse divisions. Cells, moreover, do not enlarge much between successive longitudinal divisions.

At first the procambium cells are no longer than the adjacent ground-meristem cells, but are much narrower. In a Golden Plume petiole 2.5 cm long, with the first xylem and phloem elements mature, parenchyma cells were approximately 35 microns long and 25 microns wide; procambium cells  $40 \times 6$  microns large. When the procambium is changing into cambium, ground-parenchyma cells are broader than high. They measured, in the Golden Plume plant,  $40 \times 50$  microns; the corresponding procambium cells  $60 \times 8$  microns.

The division of the procambium cells between the xylem and phloem causes a steady enlargement of the procambium strand, particularly in radial extent. At the same time, new cells are added to the strand on its periphery. In this process the ground-meristem cells surrounding the procambium stretch somewhat, perpendicularly to the periphery of the strand, then divide by a wall that is periclinal with respect to the strand. Thus shell-like layers are formed around the young procambium strand (fig. 1, *C*, left). Cells of the layers nearest the strand then divide by anticlinal walls and become part of the procambium itself.

Differentiation of primary vascular tissues begins long before the procambium strand reaches its full size. The addition of cells on the periphery of the procambium ceases, however, in regions opposite (in radial direction) the mature elements, but continues right and left from these (fig. 1, *E*). This lateral growth is very active, so that the bundle increases in width tangentially, particularly in the phloem region (fig. 1, *G*). As more phloem elements mature, left and right from the first ones, the addition of new procambium cells becomes limited more and more to the extreme margins of the two lobes of phloem. The phloem, in consequence, assumes its characteristic crescent shape (fig. 1, *G* and *H*). Plate 3 shows a bundle in which addition of new procambium cells has not yet ceased.

The first phloem and xylem elements differentiate rather close to each other (fig. 1, *E*) ; but continued division of procambial cells in the center of the bundle increases the distance between them (fig. 1, *G* and plates 3 and 4). The procambium cells formed centrifugally from the xylem differentiate into new elements of this tissue, while new phloem cells arise on the opposite side of the bundle. Since, however, the differentiation of elements progresses faster than the division of procambium cells, the xylem and phloem gradually approach each other (figs. 1, *D*, *F*, *H*, and 2, *B*, *C*.)

As the layer of dividing procambium cells narrows down to a few rows of cells (plates 3 and 4), the meristem shows an increasing similarity to the cambium of herbaceous dicotyledons. Eventually cells appear with short radial diameters and become arranged, in longitudinal sections, in horizontal tiers. The longitudinal divisions are predominantly periclinal, and the resulting cells retain a radial arrangement in the mature state.

These characteristics, however, appear in the meristem even before the petiole ceases to elongate, so that the vascular tissues produced by this meristem are morphologically primary tissues. In bundles like that shown in plate 4, for example, mature sieve tubes and vessels are longer

than their mother cells; and the vessels have spiral secondary thickenings.

Because of the terminology generally adopted in modern botanical texts, the meristem lying between the xylem and phloem is here called procambium as long as it forms primary vascular tissues. Thus bundles in plates 3 and 4, which were taken from elongating petioles, have no cambium yet.

When petioles cease to elongate, continued tangential divisions in the procambium give rise to the metaxylem and metaphloem. In their mature state the cells of these tissues show a definite radial arrangement.

Vascular bundles with primary tissues complete are shown in plates 5 and 7. Although the meristem between the xylem and phloem in these bundles has differentiated into the cambium the latter had not produced any tissues at this stage. In general no secondary tissues were present in petioles used in this study.

The early ontogeny of the vascular bundles in celery does not fully agree with the generalized description of the differentiation of primary vascular tissues given by Eames and MacDaniels (1925, p. 87). These authors state that at the beginning of differentiation of vascular tissues "the portions of procambium which form the first xylem and those which form the first phloem are situated well apart," and their figures 42 (p. 84) and 44 (p. 87) indicate that the procambium reaches its full radial extent before the first xylem and phloem differentiate. Eames and MacDaniels give no details about the course of events in the median region of the procambium strand; they merely remark on page 130 that "just before the formation of such a sheet of tissue (cambium) there is a transitional period during which cell division is taking place in various planes in the central procambial zone, but tending in later stages of development to occur more and more in the tangential plane only."

In celery the mode of development of the primary vascular bundles resembles that given by de Bary (1884, pp. 389-390) for "thick, and especially collateral bundles." In such bundles the longitudinal divisions that initiate them in primordial organs "often still go on for a long time on the boundary between phloem and xylem, when at the edges of the bundle the differentiation of the tissues is already completed."

*Phloem as a Whole.*—The development of this tissue is shown in transverse sections in figures 1, D to 2, H. The small diagrams of entire bundles accompanying the phloem sections show the relative size of the bundles from which the phloem was drawn. Quadrangles drawn in interrupted lines in the diagrams indicate the position of sections that

were drawn in detail. Some drawings and photomicrographs were made of the same bundles. Thus figure 2, *C* and *D* corresponds to plate 3; 2, *E* and *F* to plate 4; 2, *G* and *H* to plate 5.

The first protophloem sieve tubes differentiate in the upper median region of the procambium strand (fig. 1, *E*). Each sieve tube has one companion cell. The subsequent sieve tubes arise to the right and left of the first sieve tubes and also in the centrifugal direction from these. Figure 1, *G* shows this stage of development. At *obl* are some of the first sieve tubes in a crushed condition. Functioning sieve tubes are indicated by hatching and are also distinguished by their comparatively thick walls. The companion cells are brought out by dense stippling. Light stippling in certain other cells marks immature sieve tubes and companion cells.

Differentiation right and left from the obliterated elements becomes increasingly prominent (fig. 1, *H*). Then sieve tubes begin to arise centripetally from those formed earlier, while the older ones are continually crushed and obliterated (figs. 1, *I* and 2, *A* and *D*).

Phloem tissue transitional between the protophloem and the metaphloem is depicted in figure 2, *E*. The origin of this tissue from a regularly dividing meristem has become rather conspicuous. Oil ducts, which already have appeared at the stage shown in figure 2, *A*, are a regular feature of the phloem in 2, *E*.

Figure 2, *G* shows part of a bundle in which the functioning phloem is composed of metaphloem only. The sieve tubes of the protophloem have been almost completely obliterated. In the protophloem, parenchyma constitutes a prominent part of the tissue, whereas in the metaphloem, sieve tubes and companion cells predominate.

In the small bundles that have no xylem associated with the phloem (see p. 433) the first protophloem elements differentiate in the center of the procambium strand, while new elements appear uniformly in all directions from the initial ones. If any cambium-like layer is developed in these bundles, it completely surrounds the phloem and cuts off cells only toward the phloem, not toward the outside.

The sieve tubes of proto- and metaphloem, though alike in structure, differ in size. As shown by a glance at the phloem in figures 1 and 2, sieve tubes become progressively larger in their transverse diameters, so that the last metaphloem sieve tubes are the largest. Their elements are also longer than those of the protophloem sieve tubes.

The sieve tubes usually have only one companion cell when viewed in cross section, but in longitudinal sections they may have one or two companion cells (fig. 3, *C*, *D*, *F*, and *G*). In forming protophloem sieve

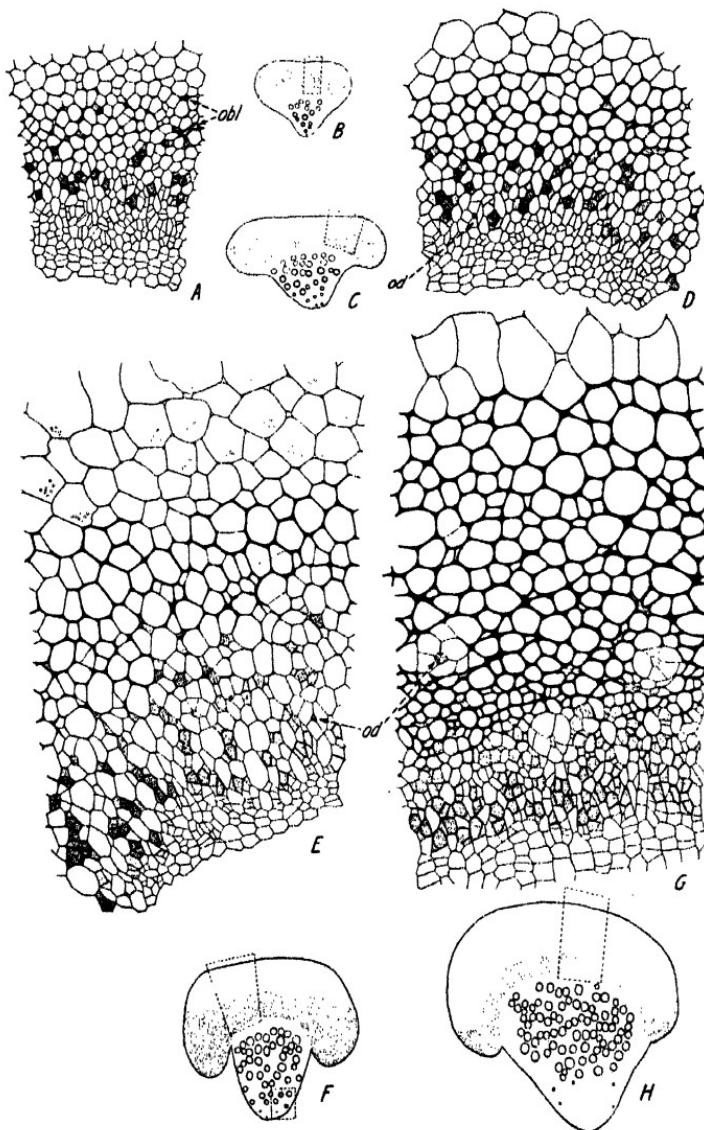


Fig. 2.—*A, D, E, G*, Transverse sections through parts of vascular bundles showing development of phloem ( $\times 158$ ); *B, C, F, H*, diagrams of entire vascular bundles from which drawings *A, B, E*, and *G* were made ( $\times 34$ ); *obl*, obliteration; *od*, oil duct.

tubes a procambium cell divides several times, the second-before-last division giving rise to a phloem parenchyma cell and a sieve-tube-companion mother cell. The latter then divides longitudinally to form a sieve-tube element and a sister cell, which may give rise to one companion cell or to more than one by dividing transversely.

The relation among sieve tubes, parenchyma, and companion cells is clearly shown in figure 3, *H* and *I*, where the groups of cells derived from one procambium cell are still clearly outlined. The sieve tubes are indicated by *st*, and the companions are the smallest cells of each group. To the left below in 3, *H*, are two phloem parenchyma cells derived from one procambium cell. Similar groupings may be found in figure 3, *M*.

In the metaphloem (fig. 3, *L*) some parenchyma cells develop from a procambium cell without division. More frequently the phloem mother cell divides by a longitudinal wall, one of the resulting cells becoming a parenchyma, the other a sieve-tube-companion mother cell; or both derivatives of the phloem mother cell give rise to sieve tubes with their companions.

In the division of the sieve-tube-companion mother cell, one of the daughter cells is larger than the other. This is the young sieve tube (fig. 3, *C*, *H*, *I*, and *M*). Besides being smaller, the companion cells are also distinguished, in a mature state, by their dense protoplasts and prominent nuclei (fig. 3, *I*, left, and *M*, above).

**Sieve Tubes.**—The ontogeny of the sieve tube follows a course similar to that of a sieve tube in the sugar beet (Esau, 1934). Figure 3, *C*–*F* illustrates four stages in sieve-tube development. The element in *D* shows increase in size and vacuolation in comparison with that in *C*. There is also a slime body above the nucleus and disk-like plastids in the cytoplasm.

The sieve-tube plastids of celery, though less prominent structures than those of the beet, are similar in shape. Plastids of celery sieve tubes, like those of many other plants, stain red with iodine.

In figure 3, *E* the walls of the sieve-tube element are thickened, vacuolation is very prominent, and the nucleus and the slime body (below the nucleus) are disintegrating. Figure 3, *G* depicts a similar stage.

A mature element is shown in figure 3, *F*. It contains a thin parietal layer of cytoplasm enclosing a large central vacuole. The plastids are included in the cytoplasm. The end walls have the characteristic sieve plates with very fine perforations. Slime accumulations are not prominent, being present only on the lower sides of the plates.

The end walls of sieve-tube elements are transverse or slightly oblique. Only one sieve plate is present on the end wall, but the longitudinal

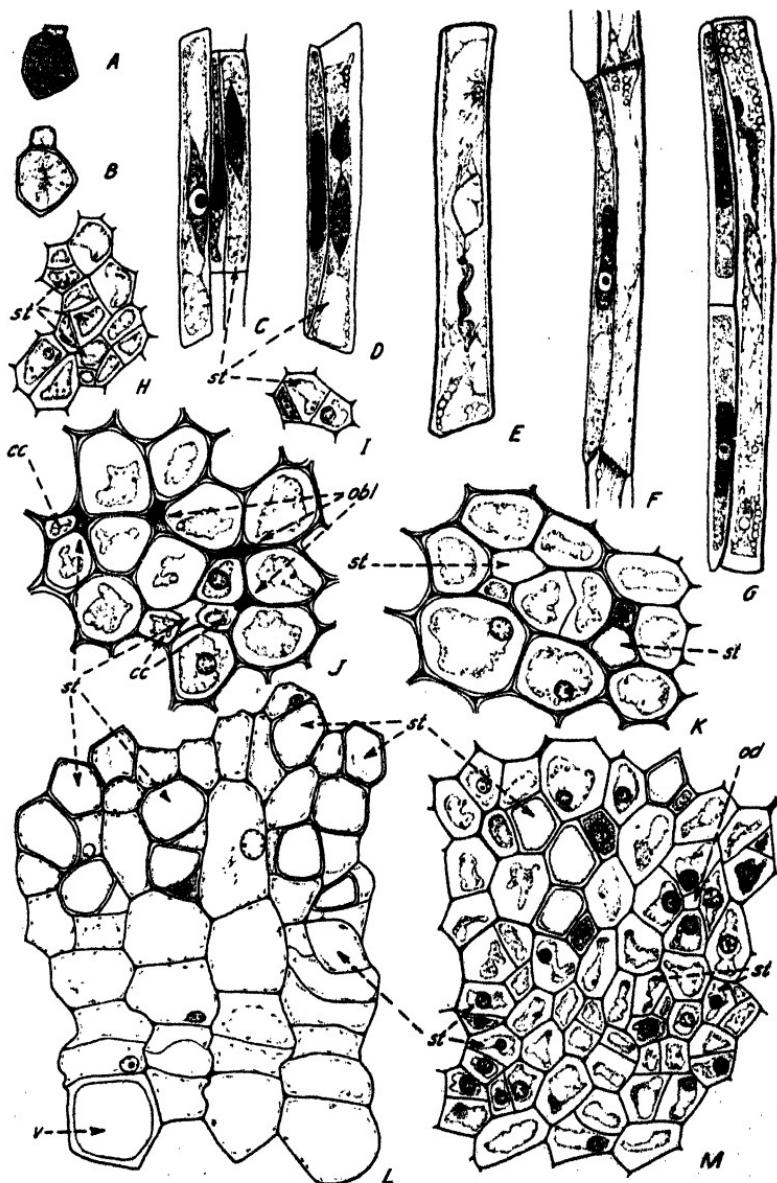


Fig. 3.—Details of phloem structure: A, B, two transverse sections of one sieve-tube element with a sieve plate at A; C-G, different stages of sieve-tube differentiation shown in longitudinal sections; H-M, different stages of phloem development shown in transverse sections; cc, companion cell; obl, obliteration; od, oil duct; st, sieve tube; v, vessel. ( $\times 700$ ).

walls have sieve fields. Figure 3, A shows a sieve plate with light-colored callus cylinders and black-stained cytoplasmic strands passing through the center of these cylinders. Figure 3, B depicts another transverse section through the same sieve-tube element as in 3, A, but at a slightly higher level. Here the thickening of the longitudinal wall is visible, and also the slime. The companion cell appears above the sieve tube in A and B.

Although the celery sieve tubes are rather small and do not represent favorable material for the study of such elements, some information was gathered on the behavior of walls in mature versus old sieve tubes in prepared sections. In early stages of differentiation the sieve tube develops thickenings on longitudinal walls (fig. 3, E). By the time the nucleus disappears and the end walls show sieve-plate perforations, this deposition of wall material is very marked; usually it appears to be of uniform thickness on all longitudinal walls (fig. 3, L and M). The wall next to the companion cell may or may not be thickened. When the sieve tube begins to approach senility the wall thickening disappears, so that a functionless element has no thicker walls than one just emerging from the mother-cell stage.

Figure 3, K shows, at the right, a functioning sieve tube with a thick wall; at the left, a functionless element with a thin wall. Figure 2 also brings out the difference in thickness of young and old sieve tubes.

Crushing of the sieve tube ensues after the loss of the thick wall (fig. 3, J, below). This crushing, accomplished by growth and expansion of adjacent parenchyma cells, continues until the lumen of the sieve tube is entirely closed. Definitive callus may be recognized on sieve plates of elements being obliterated. Companion cells also are crushed, although sometimes considerably later than the sieve tubes. Figure 3, J shows several stages in obliteration of sieve tubes and companion cells. Figure 2, G illustrates the same phenomenon at a lower magnification.

Besides the peculiarity of wall structure, the sieve tubes are distinguished by the behavior of their protoplasts in prepared sections. In young sieve-tube elements still containing nuclei, the cytoplasm shrinks as much as that of any other cell that was living at the time of sampling (fig. 3, M). In mature functioning elements, however, the cytoplasm fails to shrink and adheres closely to the walls (fig. 3, L and M, above).

A similar differential shrinkage of protoplasts in young and old sieve tubes was observed by Strasburger (1891, p. 194) in *Robinia pseudoacacia* upon treatment of the material with alcohol. Crafts (1933) found that the protoplasts of mature sieve tubes of potato phloem, in contrast to those of the young elements, cannot be plasmolyzed with

hypertonic sucrose solutions. The lack of shrinkage of sieve-tube protoplasts in prepared sections and the loss of susceptibility to plasmolytic agents in fresh sections is, perhaps, traceable to the same property of mature sieve-tube cytoplasm—its complete permeability.

*Oil Ducts.*—Among the elements of metaphloem, oil ducts are a prominent part, but they begin to develop in the protophloem (fig. 2, *A*). Gradually these structures gain in prominence (fig. 2, *D-G*). They are very conspicuous in the phloem in plate 5.

Oil ducts of the phloem, as well as those of the cortex, develop as schizogenous intercellular spaces. Figure 4, *E* and *F* shows two stages in phloem oil-duct development. In *E* the intercellular space has just made its appearance, whereas in *F* it has grown to a large cavity.

In the formation of an oil duct in the cortex, cells undergo several divisions. As a result, the mature duct is lined with many cells, whereas there are usually only three cells in contact with the cavity when the duct is initiated. (Compare oil ducts in figures 1, *C* and *E*; and 5, *A-D*). In the phloem, a similar process may take place; but frequently the intercellular space increases in size so rapidly that contact of the duct with new cells is brought about by separation of pairs of cells to their common line of union with a third cell (fig. 4, *E* and *F*).

Lining the oil duct are active cells elaborating an ethereal oil that later accumulates in the duct. These cells have prominent nuclei (figs. 3, *M* and 4, *I*) and thin walls (fig. 4, *F*; plate 5). Contrary to Sayre's statement (1929), no lignification was observed in them.

The product of the oil ducts often appears as coarsely coagulated material within the cavity. Frequently the contents of the duct stain densely (plates 4, *A* and 5).

In the sections examined the oil ducts, as far as visible, were long, continuous structures. In longitudinal view the cells lining the cavity are arranged end to end, as shown for a cortical duct in figure 4, *I*. Here the two rows of active cells to the right and left of the duct are conspicuous by virtue of their dense cytoplasm.

*Bundle Cap.*—The caps of the vascular bundles in celery arise in a manner similar to that of the bundle cap in sugar-beet leaves (Esau, 1933 and 1934). This structure is composed of phloem-parenchyma cells that have enlarged and have thickened their walls subsequent to the obliteration of sieve tubes and companion cells.

The cells of the phloem parenchyma are living. Their walls are thin in the functioning phloem (figs. 2, *A*, *D*, *E*, *G*; 3, *L* and *M*) but thick in the nonfunctioning tissue (figs. 2, *E* and *G*, above; 3, *J* and *K*). They are of nonlignified cellulose. The wall thickening is somewhat more

prominent in the corners than elsewhere, and its resemblance to the thickenings of collenchyma walls leads to the use of the term "collenchymatous bundle cap." The ontogeny of the bundle cap, however, and its mechanical properties, which are discussed later in this paper, clearly differentiate it from true collenchyma.

When first formed, the phloem parenchyma cells have transverse or slightly oblique end walls (fig. 3, C, left). Later, through readjustments, perhaps in the nature of symplastic growth (Priestley, 1930), the cells become more tapering and assume the appearance shown in plate 7, right. The horizontal walls, visible in these cells, are thin partitions formed in late stages of ontogeny when the cells have undergone some elongation. The walls of the mature cap cells are rather thick and have simple pits.

Parenchyma cells in actively growing phloem undergo marked spatial rearrangements. Certain of these cells force others apart and make new contacts. Sometimes a cell surrounded by other parenchyma cells later comes in contact with a sieve tube or a companion cell. This phenomenon, very common in celery phloem, is much like that observed in the xylem of rapidly expanding vessels (page 448).

Figures 1, G, I, and 2, A, D, E, and G show details of bundle-cap development. The phenomenon of sieve-tube obliteration is most marked in 2, G. The diagrams of entire bundles in 1, F, H, and 2, B, C, F, and H show the relative growth in size of the bundle cap, which is represented by the white area above the stippled portion. The latter includes the differentiating and the functioning phloem.

**Xylem.**—The xylem tissue in celery shows a rather simple structure, containing only vessels and xylem parenchyma. The early ontogeny of the xylem is shown in figure 1, E and G, whereas figures 1, D, F, H, and 2, B, C, F, H, together with plates 3, 4, and 5, illustrate the relative increase in the amount of xylem in progressively older bundles.

The xylem is endarch, differentiating centrifugally. The first protoxylem elements have small diameters (plate 3, below); the succeeding ones become progressively larger (plates 3 and 4). When growth of the bundle slows down, vessels smaller in diameter are again produced; but their proportion with respect to the xylem parenchyma increases.

Plates 5 and 7 show on the adaxial side of the bundle the first protoxylem with obliterated vessels. Then follows the intact part of the protoxylem. Next to the cambium appears the metaxylem, containing scalariform and reticulate elements and a small proportion of xylem parenchyma.

The majority of vessels in celery petioles have spiral secondary thick-

enings. The first vessels formed may show annular bands, but usually only in localized regions of the vessels.

The secondary walls of the xylem vessels show lignin reaction with phloroglucin and HCl.

Spirals while being formed are laid down rather closely over the primary walls, but the coils become separated first through continued longitudinal growth of elements and later, after maturation, by passive stretching caused by growth in length of the petiole. Although the scalariform and reticulate elements of the metaxylem undergo no stretching after maturation, their mother cells are longer in comparison with the procambium cells from which they were derived.

The xylem mother cell develops into a vessel element without any divisions; but it rapidly increases in width and length. The meristem cells giving rise to xylem parenchyma cells, however, undergo longitudinal and transverse divisions, so that the resulting cells are narrower and shorter than the vessel elements.

Vessel mother cells grow and expand so rapidly that the spatial relation of cells in the xylem is considerably affected. There is not only a flattening and distortion of adjacent cells, but actual tearing apart of cells, so that new contacts are made by the expanding vessel. Such phenomena have already been noted by Priestley and his co-workers (1935) in woody angiosperms.

Figures 4, C and D show two sections from the same portion of xylem, one being 10 microns below the other. Whereas in 4, C vessels *a* and *b* are separated by a parenchyma cell, in 4, D they are in contact with each other. The expanding vessel *a* has torn apart parenchyma cells that intervened between it and vessel *b*.

The separation of parenchyma cells from each other does not occur uniformly along the entire wall, and the two cells being forced apart remain in contact in several places. This situation is made possible by the growth of walls in localized regions, so that the cells appear to have arms or protuberances connecting one cell to the other.

Figure 4, G shows an early stage in the separation of parenchyma cells; 4, H a later stage in the process. Both drawings represent longitudinal sections. The lack of contact between some of the protuberances is an artifact obtained in cutting thin tangential sections off a cylindrical body, the vessel. Where protuberances are not in contact, a portion of them was sliced off with the preceding section of the paraffin ribbon.

Priestley and his co-workers (1935) suggest that the places where cells remain in contact by means of arms are the pitted places.

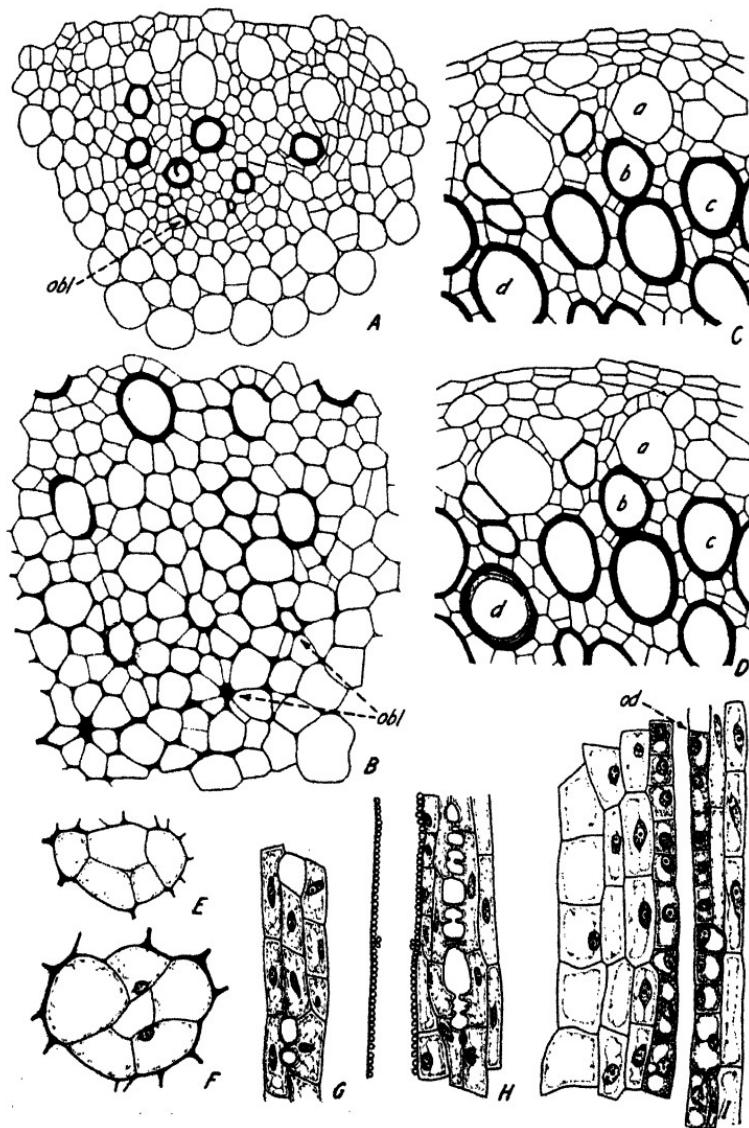


Fig. 4.—*A, B*, Two stages of protoxylem development; *C, D*, two successive sections of the same xylem region showing relation of vessels to surrounding cells; *E, F*, oil ducts from metaphloem; *G, H*, tearing apart of xylem parenchyma cells; *I*, oil duct from cortex; *A-F*, transverse sections; *G-J*, longitudinal sections; *obl.*, obliteration; *od*, oil duct. (*A-D, G and H*,  $\times 211$ ; *E, F*,  $\times 467$ ; *I*,  $\times 301$ .)

Xylem-parenchyma cells assist in obliterating the nonfunctioning vessels in the protoxylem. When a vessel becomes stretched so that its spiral is unable to keep the vessel open, adjacent parenchyma cells elongate anticlinally with respect to the vessel, thereby closing its lumen. This change in shape of parenchyma cells may be accompanied or preceded by periclinal divisions.

Figure 4, *A* and *B* shows stages in obliteration of vessels. In *A* only one of the protoxylem vessels has been obliterated at *obl*; the others are still intact, although in the smallest ones the lumina have been narrowed down. The upper part of the drawing shows some xylem mother cells without secondary thickenings. This section was taken from a bundle represented in figure 1, *H*.

In figure 4, *B*, which was drawn from the bundle represented in outline in figure 2, *F*, and which also occurs in plate 4, further progress in protoxylem obliteration may be noted. The lowest elements have been closed by the much enlarged xylem-parenchyma cells, whereas the one above and to the right has been partly crushed. Two more elements above have also been subjected to much stretching and therefore show only portions of their loose spirals. Xylem-parenchyma cells begin to encroach upon these vessels. Three larger vessels above have wide-open lumina, but their spirals are also somewhat loose and appear only partially in sections. Above, to the left, is a functioning vessel with a close spiral appearing as a ring in cross section.

Upon obliteration of protoxylem vessels, xylem-parenchyma cells thicken their walls in a manner similar to that of the bundle-cap cells. Plate 5 shows to the left the characteristic appearance of the nonfunctioning protoxylem, with parenchyma cells radiating from the points where vessels were crushed. Darkly stained material has accumulated in place of the destroyed cells. Plate 7 shows, at the left, the same region in longitudinal section. Pits in the thick walls of xylem parenchyma appear as light dots.

*Primary and Secondary Walls in the Vascular Tissues.*—In the analysis of the wall structure of vascular as well as other tissues, the recent revision of cell-wall terminology made by Kerr and Bailey has been adopted in this paper. For clarity of descriptions of walls, the following quotation is taken from the work of those authors (Kerr and Bailey, 1934, p. 342) :

There are two distinct and fundamentally different categories of cell walls. Meristematic elements and such of their derivatives as retain a potentiality for growth and enlargement have walls which are characterized by their capacity for growth and extension and for undergoing reversible changes, e.g. in thickness. On the contrary,

tissue cells which undergo irreversible changes and thus lose their potentiality for growth and enlargement may form supplementary or *secondary wall* which tends to be more or less conspicuously laminated.

The first category of walls mentioned includes the primary walls.

Applying this classification to walls in celery petioles, we find that the only elements showing secondary walls are the vessels. The walls of the phloem parenchyma, even after the latter is transformed into the bundle cap, must be classed as primary, because its cells are living and can undergo reversible changes. In connection with sieve-tube obliteration the parenchyma cells continue to grow and divide after their walls have been thickened. In the sugar beet (Esau, 1933) bundle-cap cells lose their wall thickenings when anomalous cambium is formed in the bundle cap.

The sieve tubes develop rather thick walls when they emerge from the mother-cell stage, but the thickening disappears again when the elements become senile. Because of their transitory nature, sieve-tube wall thickenings are interpreted as primary, pending more detailed studies.

The xylem parenchyma develops rather thick walls in the old protoxylem regions, but these walls are similar to those of the bundle-cap cells. Also like the latter, xylem parenchyma cells are living in their mature state.

Vessels, however, have secondary walls. After these appear, the cells lose their protoplasts and become incapable of reversible changes.

It is beyond the scope of this investigation to attempt to differentiate between the primary wall and the intercellular substance (Kerr and Bailey, 1934).

#### ONTOGENY AND STRUCTURE OF THE COLLENCHYMA

*Ontogeny of Collenchyma.*—The collenchyma in the ribs of celery petioles is of a type in which the major portion of the wall material is deposited in the corners of cells (plate 6).

As was mentioned earlier in this paper (p. 436), collenchyma arises within the ground meristem, between the oil duct and the protoderm. This part of the ground meristem develops intercellular spaces before the divisions initiating collenchyma become evident.

Figure 5, A, depicting the initiation of collenchyma, shows that periclinal divisions predominate at first but are soon followed by anticlinal longitudinal divisions. The subepidermal layer does not at once take part in these active divisions. Eventually, however, it contributes some cells to the collenchyma by cutting off tangentially a row of cells toward the collenchyma strand (fig. 5, B, C, and D). The resulting cells of the outer layer then grow as large as the subepidermal cells of regions where

no collenchyma is present, and remain as a transitional layer between the collenchyma and the epidermis. This differential growth of the subepidermal cells is shown successively in plates 3; 4, A, and 6.

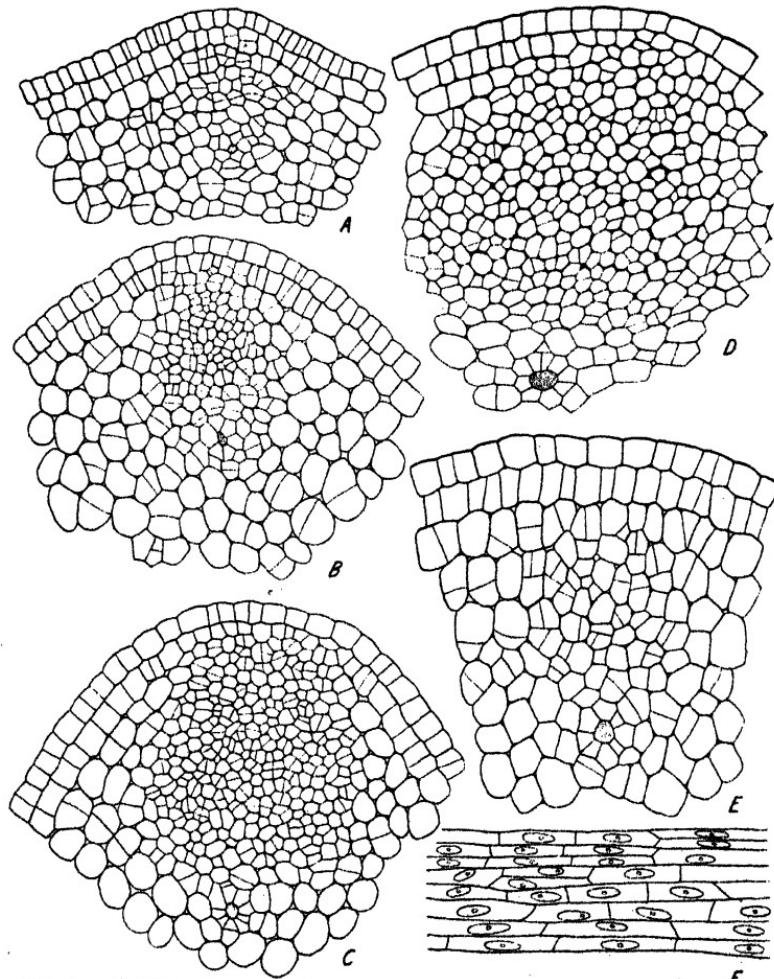


Fig. 5.—A-E, Transverse sections of collenchyma ( $\times 215$ ); A-D, four successive stages of development; E, late initiation of collenchyma; stippled cells representing oil ducts; F, young collenchyma in longitudinal view. ( $\times 307$ .)

Growth of the young collenchyma strand somewhat resembles the growth of a procambium strand. The early divisions lead to the formation of a strand of elongated cells of small diameters. This strand continues to grow through repeated divisions within the strand itself and

through the addition, on the periphery, of new cells derived from the ground meristem (fig. 5, A, B, and C).

Divisions follow one another so rapidly that cells enlarge very little from division to division and soon appear much smaller than the ground-parenchyma cells about them (fig. 5, A, B, and C). Only when divisions slow down do collenchyma cells begin to enlarge (fig. 5, D).

The rapid succession of divisions in the young collenchyma strand causes a close packing of cells and loss of those intercellular spaces that were present among the initiating ground-meristem cells. Intercellular spaces occur among recently divided cells in figure 5, A, but are limited to the peripheral layers of collenchyma in 5, B. Perhaps the deposition of wall material in the corners aids in closing intercellular spaces along with the packing of dividing cells. The collenchyma strand in plate 7 has no intercellular spaces. The small lumina visible in the photograph are cross-sectional views of the tapering ends of collenchyma cells.

Although the deposition of the characteristic wall thickenings begins in very early ontogeny of collenchyma (fig. 5, B), it does not make much progress until cell division slows down (fig. 5, D).

Meristematic collenchyma cells are longer than wide and have transverse or somewhat oblique end walls (fig. 5, F). While increasing in length, collenchyma cells develop tapering end walls, probably through processes akin to that termed by Priestley (1930) symplastic growth. Some thin transverse walls develop in the later stages.

Plate 8 shows the long tapering ends of mature collenchyma cells and the thin transverse partitions. No pitting is evident. It seems to be limited to the thin portions of walls.

Some collenchyma strands are initiated later than others, in a more mature region of the petiole. Figure 5, E shows origin of such a collenchyma strand. It was taken from the same transverse section of the petiole as 5, D. As the intercellular spaces are large when a bundle of this kind is initiated and as divisions are less rapid than in younger regions, intercellular spaces are frequently retained in the mature strand.

Using Kerr and Bailey's terminology (1934), the wall thickenings of collenchyma should be considered as primary. Collenchyma cells are living, retain a potentiality for growth and enlargement; their walls have a capacity for undergoing reversible changes, as evidenced in the case of phellogen formation in collenchyma of woody species.

*Chemical Nature of Collenchyma Walls.*—The walls of collenchyma are chiefly of cellulose and contain a high percentage of water. Anderson (1927) was able to show the presence of pectic materials as well as of cellulose, and the increase of pectic materials in amount toward the

"middle lamella." Using agents that dissolve out the pectic substances, he has found that cellulose and the pectic substances alternate in layers. The collenchyma walls are doubly refractive in polarized light, before and after removal of pectic substances.

Cohn (1892) has attempted to determine the amount of water in collenchyma walls, using the following procedure. He carefully peeled out the strands of collenchyma, soaked them in water for 24 hours, then weighed them after having removed excessive moisture with filter paper. After drying the strands for some time at 95°–100° C, he weighed them again; and from drawings of their cross sections he obtained the ratio between walls and lumina. He also determined the specific weight of cellulose needed for calculating the volume of wall substance. Assuming that the lumina of cells are filled with water, he arrived at values of 60 to 75 per cent of water in fresh weight of collenchyma walls. In contrast, he found that walls of wood from several different species of trees contained approximately 25 per cent water, those of the lignified "bast" of *Vitis vinifera* 34 per cent, of maple 39 per cent. He also ascertained that, after heating, collenchyma walls lost their ability to absorb moisture and became in this respect like fibers.

By examining under a microscope the behavior of collenchyma walls upon addition of dehydrating agents, Cohn learned that the transverse contraction of walls was much more pronounced than the longitudinal. The values obtained for collenchyma of *Eupatorium cannabinum* were as follows: contraction in radial direction 27 per cent, in tangential 10 per cent, and in longitudinal  $\frac{1}{2}$  to  $\frac{3}{4}$  per cent.

Anderson (1927) suggests that alternation of layers of cellulose and pectin explains the strong transverse contraction, the pectic material being capable of holding large amounts of water. Heating destroys the colloidal nature of pectic substances and makes them unable to absorb large amounts of water again.

#### MECHANICAL STRENGTH OF COLLENCHYMA AND OF THE VASCULAR TISSUES

In view of Sayre's (1929) statement that collenchyma is apparently the only tissue having a definite relation to stringiness in celery, an attempt was made to compare the strength of collenchyma with that of the vascular tissues.

Sayre reports that collenchyma in tough, stringy specimens is especially hard, judging by its tendency to break under the microtome knife. To support his contention, he gives several photomicrographs with broken and unbroken collenchyma. His figure 3, however, shows con-

siderable tearing in the xylem also; in figure 6 the bundle cap and phloem are badly torn; and in figure 10 even the soft ground parenchyma is seriously damaged. Moreover, the breaking of collenchyma often occurred in streaks, leaving other parts of the same collenchyma strand intact (Sayre, figs. 10 and 13). Such irregular tearing must have been caused by a defect in the microtome knife.

By proper technique, tearing may be reduced to a minimum. In this study, collenchyma strands showed very little tearing (plates 2, 3, 4, 6, and 8), even in old petioles of very stringy varieties, and offered less difficulty in cutting than the xylem. Evidently, therefore, the degree of breaking of collenchyma in paraffin material is not a reliable measure of its toughness. As will be shown, however, Sayre is correct in assuming that collenchyma is tougher than the vascular strands.

Experiments to measure toughness of celery were made in 1881 by Ambronn, who used a method similar to Schwendener's (1874) for determining elasticity of fibers. A strand of tissue was broken by fastening one end of it with a clamp and suspending weights to the other end. By means of a scale with a pointer, brought in motion by the added weight, the elongation of the strand could be determined. Having made drawings of cross sections of strands, Ambronn determined the area occupied by walls and calculated the tensile strength of the tissue.

Ambronn found that collenchyma could support 10 to 12 kilograms per mm<sup>2</sup>, values that compare very favorably with the 15 to 22 kilograms per mm<sup>2</sup> obtained by other workers for fibers. The latter structures are, however, more elastic than the collenchyma, because they regain their original length even after having been subjected to a tension of 15 to 20 kilograms per mm<sup>2</sup>, whereas collenchyma cells pass their elastic limit at 1½ to 2 kilograms per mm<sup>2</sup>. Fibers elongate 1.5 per cent of their original length before they break; collenchyma strands 2 to 2½ per cent. From his data Ambronn concludes that as a supporting tissue collenchyma is particularly adapted to growing parts of plants. Its toughness offers firm support; but its low elasticity, combined with an ability to respond to tension by stretching, prevents it from hindering the elongation of growing organs.

In the present study the strength of collenchyma and vascular bundles was compared by determining the breaking load of individual strands of these tissues.

Strands of collenchyma or of vascular tissue were peeled out of fresh turgid petioles. With care, one can obtain strands several centimeters long, almost free of adhering parenchyma. The vascular bundles were used entire or were separated along the cambium into bundle cap and

xylem. The bundle cap, of course, included the functioning phloem; but to simplify description it is called simply the bundle cap. Cells of the bundle cap undoubtedly determine the strength of the abaxial part of the vascular bundle, the phloem being a soft thin-wall tissue.

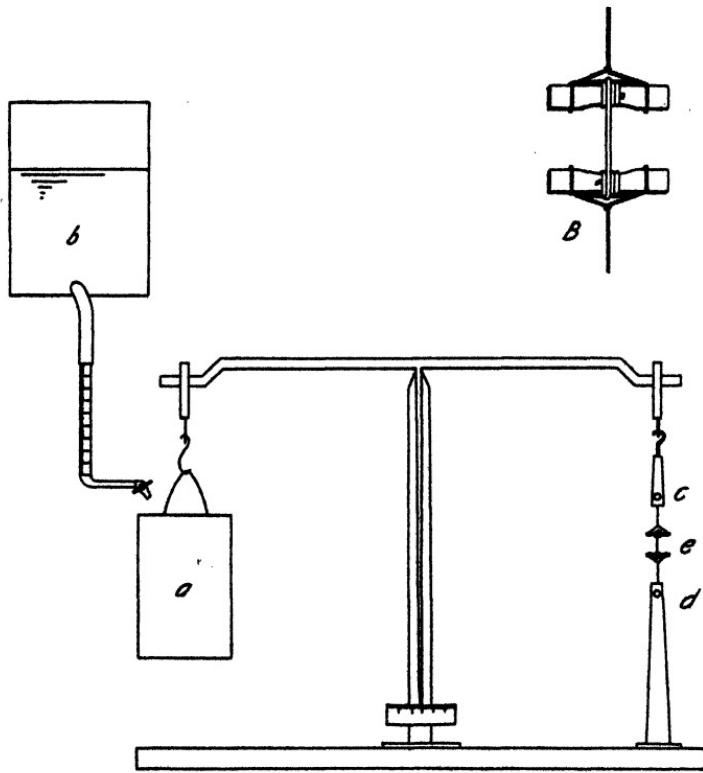


Fig. 6.—*A*, Apparatus used in determining breaking load of collenchyma and vascular bundles; *B*, detailed diagram showing method by which strands of tissue are attached.

To keep the strands fresh, they were placed in petri dishes on moist filter paper and were taken out one by one.

The apparatus to measure the breaking load consisted of a specially adapted balance shown in figure 6, *A*. On the left arm, container *a* for water was suspended directly under a burette leading from a stationary container *b* with the stock supply of water. On the right arm, clamp *c* was suspended above the stationary clamp *d*.

The celery string at *e* was not directly clamped into *c* and *d*, because

TABLE 2  
RELATIVE STRENGTH OF COLLENCHYMA AND OF THE VASCULAR BUNDLES

Variety	Leaf No.	Breaking load in grams				Average area in mm <sup>2</sup>			
		Collen-chyma	Vascular bundle	Bundle cap	Xylem	Collen-chyma	Vascular bundle	Bundle cap	Xylem
Golden Plume	13	730	185	...	...	0.471	0.522	...	...
		1,000	215	...	...	0.978	0.805	...	...
		1,020	250	...	...	1.330	0.747	...	...
	20	1,210	...	355	260	0.532	...	0.804	0.491
		1,300	...	240	95	0.905	...	0.616	0.322
		1,500	...	230	140	0.583	...	0.613	0.413
		1,500	...	265	150	0.951	...	0.638	0.414
	22	1,205	275	...	...	0.960	0.584	...	...
		1,300	...	275	155	0.785	...	0.904	0.400
		1,315	525	...	...	0.976	0.983	...	...
		1,340	390	...	...	1.046	0.910	...	...
	24	1,525	365	...	...	0.995	1.111	...	...
		1,170	...	380	170	0.475	...	0.581	0.328
		1,275	...	290	225	0.407	...	0.558	0.348
		1,500	...	340	150	0.491	...	0.469	0.316
Tall Golden Self-blanching	20	720	305	...	...	0.413	0.558	...	...
		720	390	...	...	0.442	0.721	...	...
		940	500	...	...	0.305	0.796	...	...
	21	800	...	190	95	0.266	...	0.380	0.266
		1,100	...	265	130	0.396	...	0.417	0.324
		1,180	...	255	130	0.326	...	0.408	0.338
		1,200	...	210	130	0.427	...	0.350	0.314
	22	800	...	210	80	0.291	...	0.320	0.261
		850	320	...	...	0.372	0.542	...	...
		880	405	...	...	0.301	0.632	...	...
		880	440	...	...	0.368	0.695	...	...
		890	240	...	...	0.367	0.504	...	...
		940	260	...	...	0.304	0.729	...	...
Utah	11	420	164	...	...	0.238	0.628	...	...
		425	300	...	...	0.202	0.775	...	...
		505	216	...	...	0.246	0.541	...	...
	12	305	122	...	...	0.253	0.316	...	...
		430	222	...	...	0.217	0.728	...	...
		500	204	...	...	0.227	0.677	...	...
	13	540	300	...	...	0.362	0.852	...	...
	24	1,325	375	...	...	0.411	1.116	...	...
		1,350	390	...	...	0.308	1.134	...	...
		1,700	475	...	...	0.359	1.253	...	...

then it would either slip out or break at one or the other points of attachment; but it was fastened as shown at *B* in figure 6. Each end of the strand was wrapped around a small bar of hardwood, made very smooth where it would come in contact with the strand. Care was also exercised in laying the last winding of the strand over the underlying coils of the same kind of tissue. The bars were then placed in wire loops, and these loops were clamped by their free ends into *c* and *d* of *A* in figure 6. The exposed length of the strand was approximately 1.5 cm, but the entire strand was 12 to 15 cm long. It is difficult to fasten shorter strands securely.

This method of fastening the strands proved fairly satisfactory. Although they broke sometimes in the middle, sometimes near one or the other end, this behavior did not seem to increase the variability of values obtained. The determinations were rejected, however, when the string broke at points of contact with the wood.

After the strand had been secured in position, water was poured into *a* at an approximate rate of 200 cc per minute. The flow was discontinued as soon as the strand snapped. The volume of water in *a* was measured, and the value obtained was later expressed in grams.

Table 2 shows the results of determining the breaking load for collenchyma and vascular tissue in different petioles of different varieties. In every case the value for collenchyma occurs in the same line with the value or values for the vascular bundle that was lying on the same radius with the collenchyma strand.

The average area of the strand represents the average between the areas at its base and at its apex. Although this area does not exactly correspond to the area in the median region of the bundle or in the region where the strand broke, it indicates the comparative size of strands.

In view of the exploratory nature of the experiment, the tensile strength was not determined in this study. The wall area at the breaking point of the strand would have had to be measured in tensile-strength calculations. This task would have presented difficulties as regards the heterogeneous tissues in the vascular bundles.

As table 2 shows, collenchyma strands are much tougher than the xylem, or the bundle cap, or the two tissues taken together. A comparison of the average areas indicates that the difference in the breaking-load values for collenchyma and vascular tissues is not determined by a difference in size of the strands of these tissues. The collenchyma strands were often thinner than the entire vascular bundles and even thinner than bundle caps taken alone. Where the collenchyma strands

were thicker than the strands of vascular tissues the difference in size was not proportionate to the difference in toughness.

The strength difference of collenchyma of two of the plants did, however, depend on the size of strings, those of the Tall Golden Self-blanching being thinner and weaker than those of the Golden Plume.

TABLE 3

CHARACTERISTICS OF COLLENCHYMA AND BUNDLE CAP SHOWN IN FIGURES 7 AND 8

Variety	Leaf No.	Collenchyma			Bundle cap		
		Figure No.	Per cent of area occupied by wall	Size of strand, mm <sup>2</sup>	Figure No.	Per cent of area occupied by wall	Size of strand, mm <sup>2</sup>
Golden Plume . . .	26	7, A	23.4	0.754	7, D	21.1	0.506
	26	7, B	35.1	0.671	7, E	26.1	0.516
	26	7, C	52.3	0.232	7, F	26.6*	0.351
	24	7, G	21.3	1.142	7, J	10.4	0.780
	24	7, H	35.9	0.589	.....	.....	.....
	24	7, I	43.7	0.403	.....	.....	.....
	23	7, M	21.1	0.713	7, K	19.4	0.496
	23	7, N	20.4	0.770	7, L	14.6	0.336
	12	7, O	25.4	0.491	7, Q	21.7	0.404
	12	7, P	40.6	0.325	7, R	22.2	0.925
Tall Golden Self-blanching . . .	24	8, A	38.4	0.207	8, C	32.0	0.325
	24	8, B	44.0	0.356	8, D	25.6	0.351
	16	8, E	45.4	0.191	8, G	29.8	0.227
	16	8, F	57.0	0.088	8, H	25.7	0.072
	12	8, I	36.1	0.114	.....	.....	.....
	12	8, J	34.3	0.186	.....	.....	.....
	12	8, K	35.2	0.181	.....	.....	.....
	12	8, L	42.1	0.041	.....	.....	.....
	23	8, M	56.4	0.227	8, P	21.8	0.504
	24	8, N	46.1	0.330	8, Q	21.3	0.589
Utah . . .	14	8, O	30.6	0.310	.....	.....	.....
	10	8, R	34.9	0.165	.....	.....	.....

The collenchyma strands of the old leaf (No. 24) of Utah, though small, were very tenacious.

Collenchyma of the younger leaves, compared with that of the older ones, showed a greater reduction in breaking-load values than the corresponding vascular bundles. The increase in toughness with age of tissue probably results from an increase in thickness of cell walls.

Though xylem strands were weaker than the corresponding bundle caps, this difference was largely determined by their relatively small size.

All the data given above are, of course, far too limited to permit any conclusions regarding the relative stringiness of different celery varieties.

The difference in the physical properties of collenchyma and the

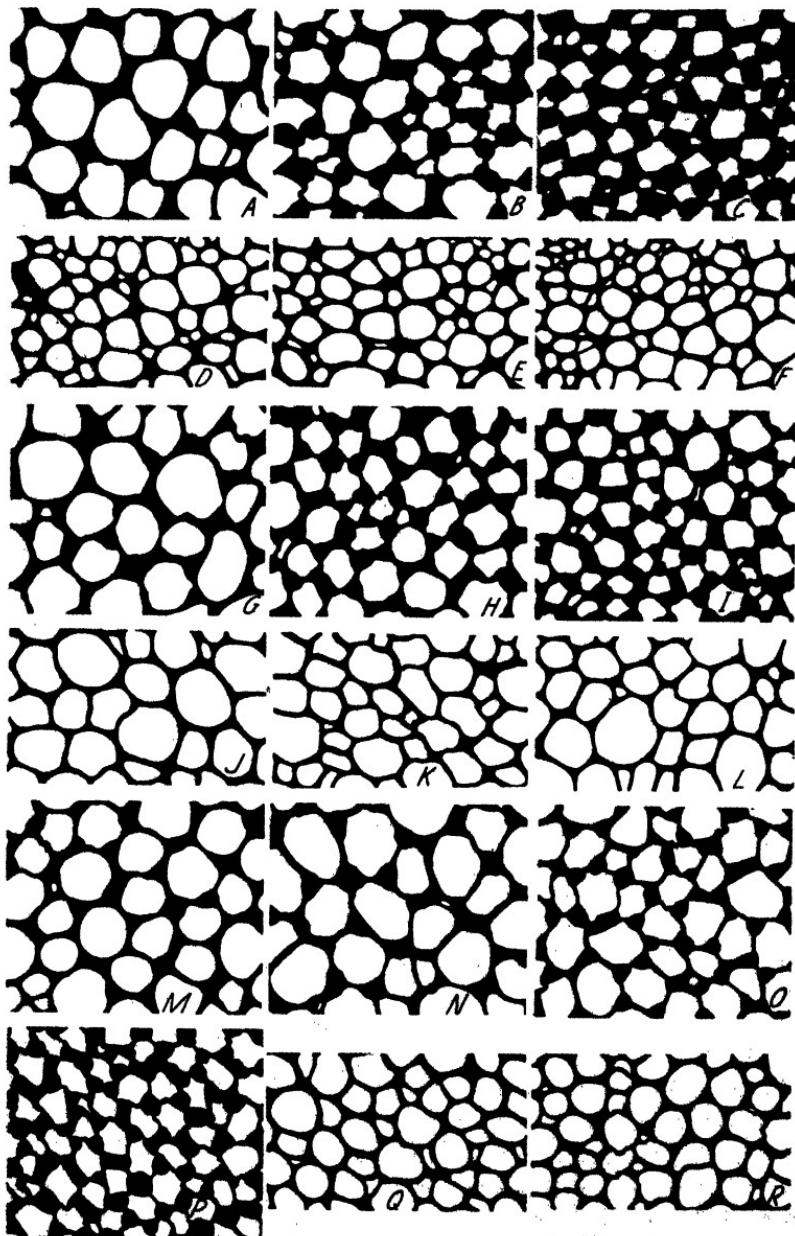


Fig. 7.—Transverse sections of collenchyma (*A-C, G-I, M-P*); and bundle cap (*D-F, J-L, Q-R*) from fresh petioles. Further explanations in table 3. ( $\times 205$ .)

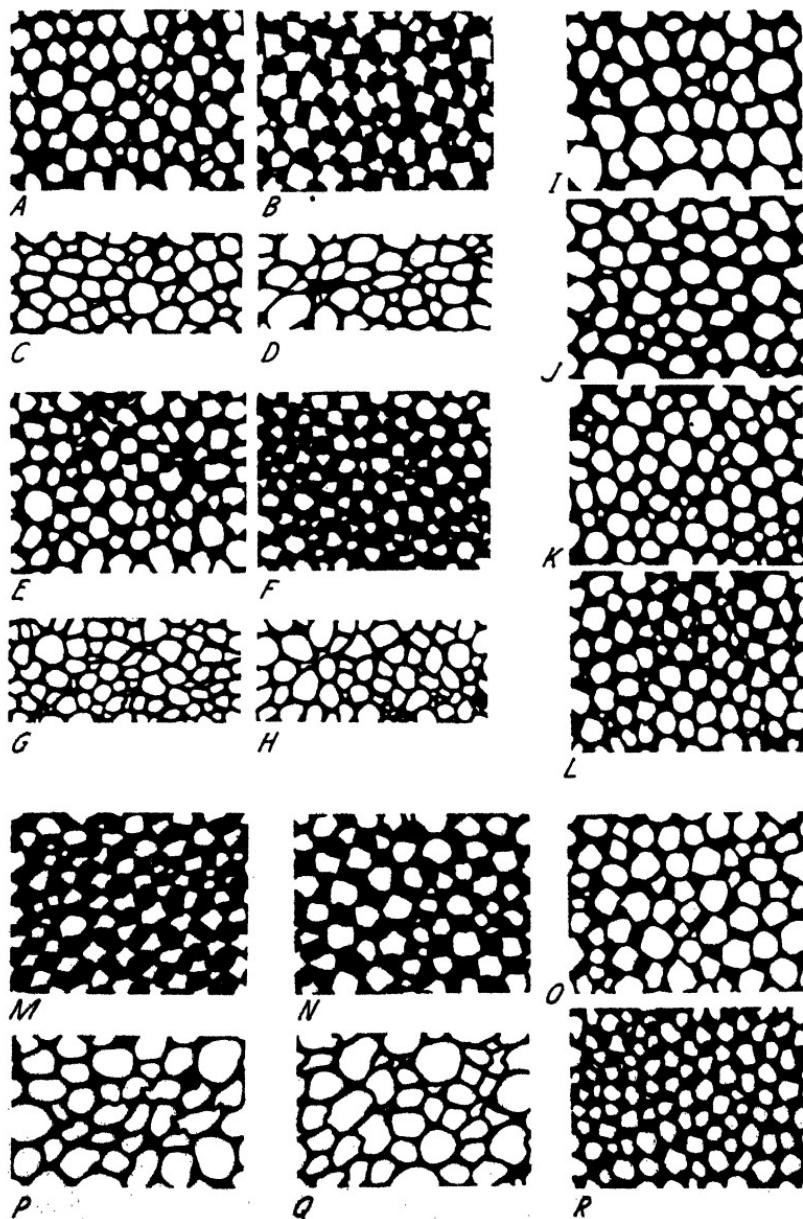


Fig. 8.—Transverse sections of collenchyma (*A*, *B*, *E*, *F*, *I-O*, *R*) ; and bundle cap (*C*, *D*, *G*, *H*, *P*, *Q*) from fresh petioles. Further explanations in table 3. ( $\times 205$ .)

bundle cap, tissues that have certain morphological characteristics in common, is, however, worthy of note. The difference in toughness between these two tissues might partly be explained by the difference in wall thickness.

Figures 7 and 8 compare collenchyma and bundle-cap structure in camera-lucida drawings from fresh sections of petioles. Table 3 gives the origin of each section, the size of the strand from which it was drawn, and the percentage of area occupied by walls in each case. This last value was obtained by measuring the reflection of light from each drawing by means of a photoelectric cell.

Figures 7 and 8 and table 3 clearly show that collenchyma has considerably thicker walls than the bundle cap and in most cases has more wall material per unit area. They also show that while the bundle cap is fairly uniform in structure in different bundles of the same petiole, the collenchyma varies markedly in cell size and wall thickness. The smaller collenchyma strands tend to have smaller cells and thicker walls. This relation is particularly obvious in the material from the Golden Plume plant.

#### DISCUSSION

The two kinds of structures commonly called "celery strings" differ greatly in their ontogeny, structure, and mechanical properties. One of these structures, the collenchyma strand, is made up of a very tough tissue which, to be broken by pulling, requires two to four times as heavy a load as the other structure, the vascular bundle.

Considering the known facts about the structure of tissues tested in this study for their toughness, we find some histological characteristics that might account for the observed differences in strength.

The collenchyma has the appearance of a strong tissue having long overlapping cells with thick walls. The xylem, for the most part, is composed of spiral vessels and thin-wall parenchyma with transverse end walls. The coils of the spirals in the vessels are separated from each other by a thin primary wall. No fibers occur in the xylem. The phloem, a living parenchymatous thin-wall tissue with no fibers, is probably, next to the cambium, the weakest tissue in the bundle. The cap cells, although prosenchymatous in shape like the collenchyma cells, are phloem-parenchyma cells that acquire thick walls rather late in their ontogeny. These walls, moreover, are not so thick as those of the collenchyma.

From the histological point of view the strength difference between collenchyma and the bundle cap is particularly interesting. Bundle

caps of the type found in celery are often called "collenchymatous structures" or even simply "collenchyma." As ontogenetic studies reported in this paper clearly show, these two types of tissues have a different mode of development. They differ in their mature structure as well as in their mechanical properties.

A consideration of the relative toughness of strings in different varieties of celery is beyond the scope of this study. A few remarks, however, should be made here regarding the possible value of breaking-load determinations in comparing varieties for stringiness. Resistance to a pulling force may not be the only measure of stringiness of a variety. Conceivably, other characteristics might play a more prominent rôle than toughness, or at least an equal rôle, in determining the brittleness of strings still imbedded in the other tissues of the petiole. One characteristic of this sort appears to be the turgidity of the tissues. Celery petioles with strong strings will appear stringless when broken after they have been cooled. Possibly, under natural conditions, celery varieties differ with regard to tissue tensions. In the Utah plant used in this experiment, the strings removed from the plant coiled like springs; but in other varieties they remained straight. This behavior, apparently, indicates that in the sample of Utah variety the strings were under greater tension within the plant than in the other varieties.

Ambronn (1881) has found that collenchyma is subjected within plants to tensions equal to 9 to 12 atmospheres, this tension being brought about by the turgor pressure of the surrounding growing parenchyma cells. Whether the vascular bundles are under similar tension has not been determined.

In studying comparative stringiness of celery varieties, one should consider the problem of tissue tensions along with the relative toughness of tissues. One should also seriously consider the fact that environment greatly influences the structure of plants, particularly with regard to the amount of wall material deposited by the cells.

#### SUMMARY

Celery petioles contain a semicircle of large collateral vascular bundles on the abaxial side and a row of very small bundles on the adaxial side. A subepidermal collenchyma strand occurs in the rib opposite each major vascular bundle on the abaxial side. Both the collenchyma and the large vascular bundles constitute the "celery strings" of growers and consumers.

The petiole of a primordial leaf contains the following meristems: the protoderm, the procambium, and the ground meristem. The protoderm

develops into the epidermis. The ground parenchyma and the collenchyma come from the ground meristem, but the collenchyma is initiated within the ground meristem through longitudinal divisions resulting in the formation of a strand similar to the procambium.

The procambium differentiates into primary phloem and xylem and the cambium. It arises in the ground meristem of the primordial petioles and increases in size partly through continued division of the first-formed cells, partly through addition of new cells from the ground meristem.

The first protoxylem and protophloem elements lie rather close together in the primordial bundles; but as the procambium cells between them continue to divide, they are moved farther apart. Through the division of the procambium cells the bundle grows in radial extent while differentiation is in progress. The division of the procambium cells increases in regularity; and as the periclinal walls predominate, the last-formed vascular tissues (metaphloem and metaxylem) show regular radial arrangement of cells.

The vascular bundles usually contain only primary tissues, although cambium differentiates between the xylem and phloem in the old leaves.

The protoxylem is endarch and is composed of spiral vessels and parenchyma. The vessels of the metaxylem have scalariform and reticulate secondary thickenings. No fibers occur in the xylem.

Differentiating vessels expand so rapidly that they tear the adjacent parenchyma cells apart and come in contact with new cells. Protoxylem vessels, which mature in rapidly elongating petioles, undergo much stretching; eventually their lumina are closed by the adjacent parenchyma cells.

The phloem contains sieve tubes, companion cells, and phloem-parenchyma cells. Slime bodies occur in differentiating sieve tubes, one in each element. Mature sieve tubes have no nuclei, but contain cytoplasm and plastids. A single sieve plate occurs on the transverse or the somewhat oblique end wall. The sieve tubes and their companion cells develop in rapid succession, the old elements being obliterated.

Phloem parenchyma cells of the old phloem continue to enlarge for some time and develop thick primary walls. When fully developed they constitute the structure known as the bundle cap.

When collenchyma is initiated in the ground meristem, the divisions occur in rapid succession so that the new cells remain small and become closely packed. The collenchymatous wall thickenings appear long before the cells cease to elongate. Mature collenchyma cells are prosenchymatous in nature, being long, with tapering ends.

Mechanically the collenchyma is much stronger than the vascular tissue. The breaking load of collenchyma may be two to four times that of the entire vascular bundles or the bundle cap.

#### ACKNOWLEDGMENTS

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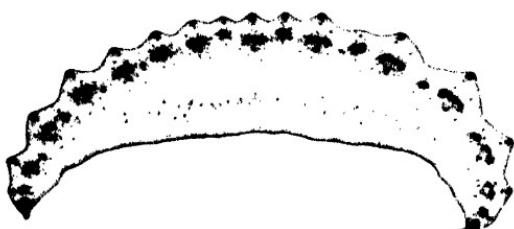
*A**B*

Plate 1.—*A*, Transverse section through a young petiole near its base; *B*, similar section through a somewhat older petiole. ( $\times 11$ .)

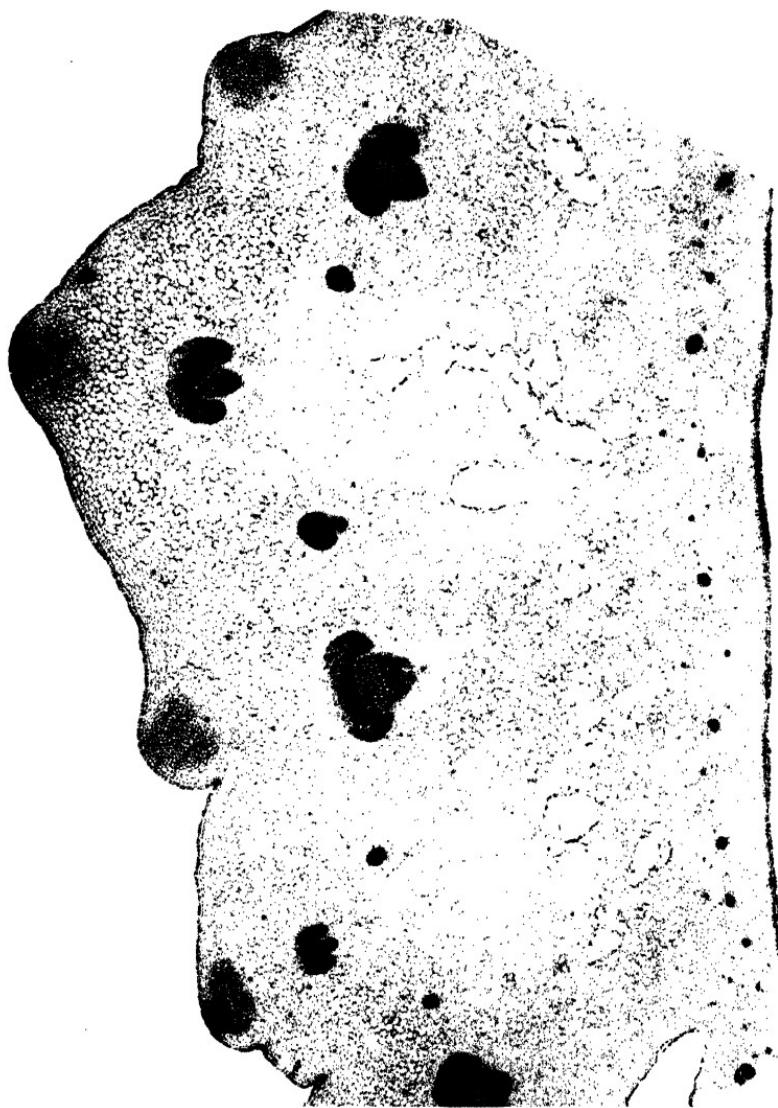


Plate 2.—Transverse section through a petiole, more mature than those in plate 1. ( $\times 11$ .)

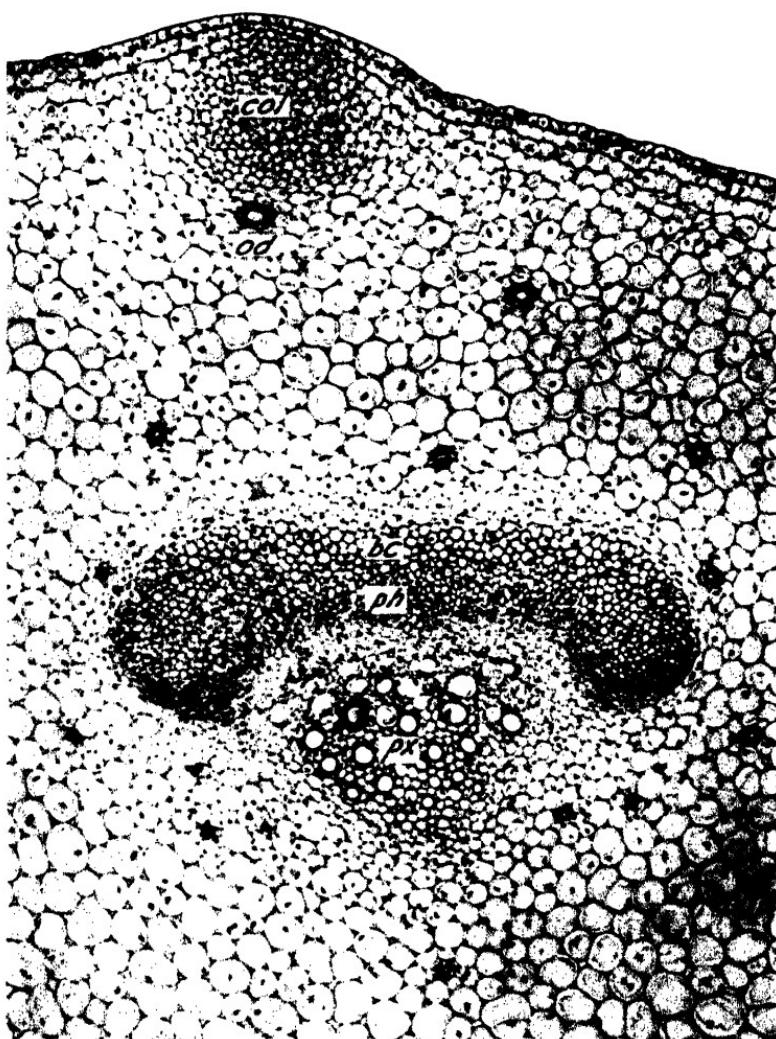


Plate 3.—Transverse section through a portion of a petiole showing a comparatively young vascular bundle and, above it, near the epidermis, a collenchyma strand. *be*, bundle cap; *col*, collenchyma; *ph*, phloem; *px*, protoxylem. ( $\times 90$ .)

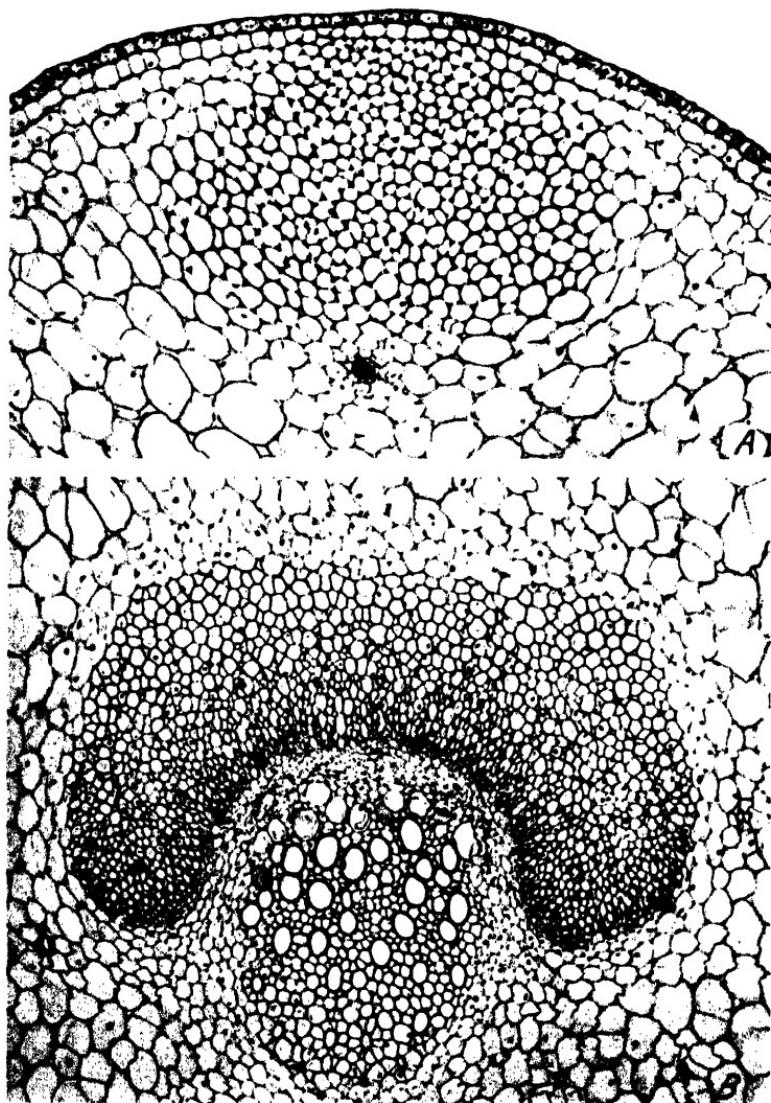


Plate 4.—Transverse sections of collenchyma (A), and of a vascular bundle (B), from a more mature petiole than in plate 3. ( $\times 90$ .)

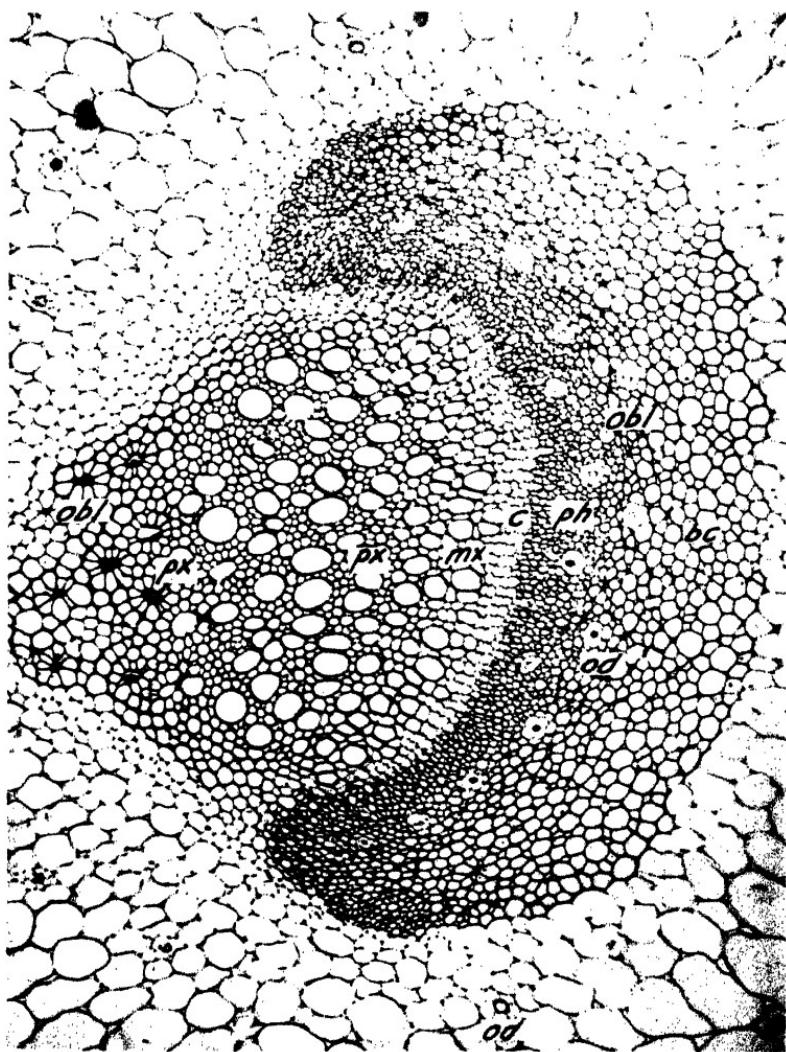


Plate 5.—Transverse section of a vascular bundle from a petiole that had ceased to elongate. The xylem is at the left; the phloem and bundle cap are at the right. *bc*, bundle cap; *c*, cambium; *mx*, metaxylem; *obl*, obliterated elements; *od*, oil duct; *ph*, phloem; *px*, protoxylem. ( $\times 90$ )

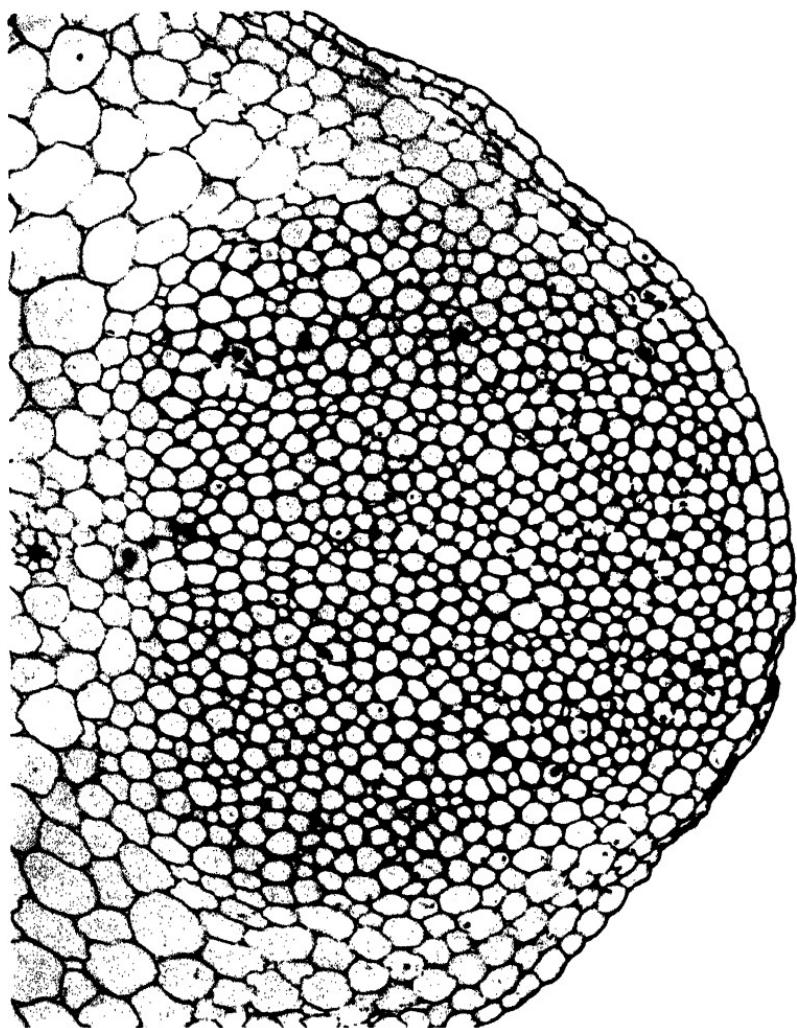


Plate 6.—Transverse section of a collenchyma strand from a petiole that had ceased to elongate. ( $\times 90$ .)



Plate 7.—Longitudinal section of a vascular bundle at a stage of development similar to that in plate 5. Tissues from left to right: parenchyma, old protoxylem (*px*), functioning protoxylem (*px*), differentiating metaxylem (*mx*), cambium (*c*), phloem (*ph*), bundle cap (*bc*), and parenchyma. ( $\times 90$ .)

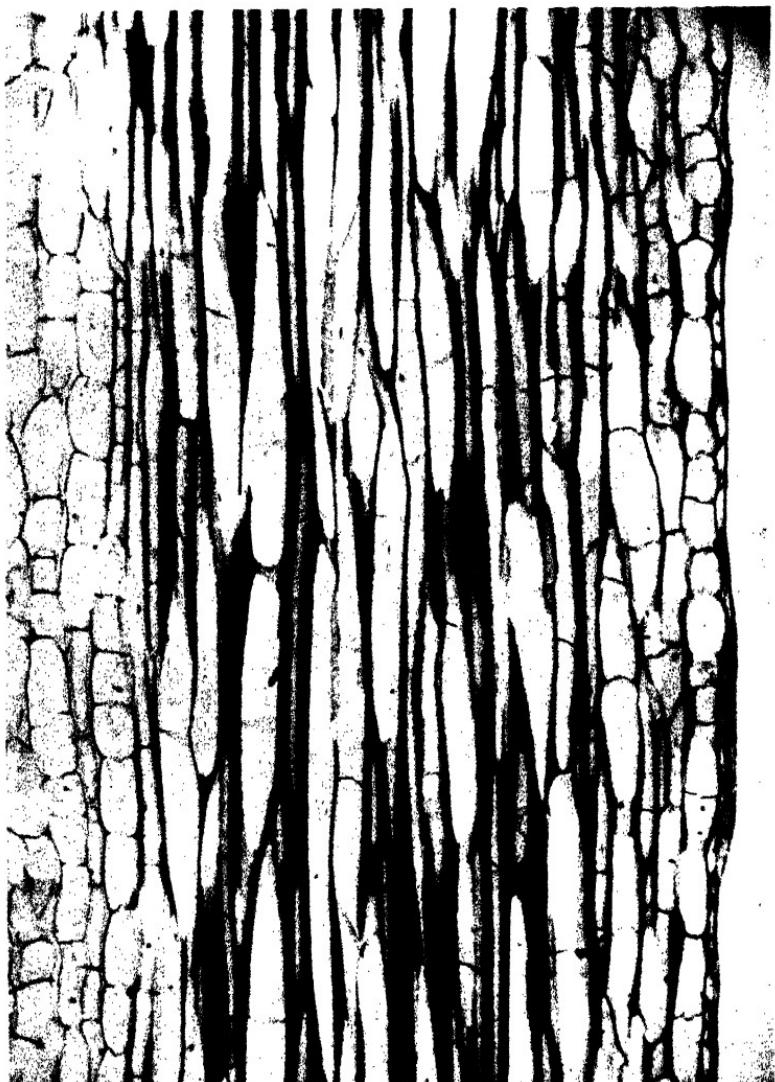


Plate 8.—Longitudinal section of a collenchyma strand at a stage of development similar to that in plate 6. ( $\times 90$ .)

# **VESSEL DEVELOPMENT IN CELERY**

**KATHERINE ESAU**



# VESSEL DEVELOPMENT IN CELERY<sup>1</sup>

KATHERINE ESAU<sup>2</sup>

## INTRODUCTION

BOTANICAL LITERATURE contains comparatively little information concerning the development and breakdown of end walls in vessels, and, moreover, the available descriptions show a surprising lack of agreement with regard to the sequence of events in the establishment of continuity between vessel elements. This circumstance prompts the writer to report the observations on vessel end walls that were made incidentally to an earlier anatomical study (Esau, 1936)<sup>3</sup> on petioles of the celery (*Apium graveolens* L.).

As in the previous investigation, the material was killed in a chromoacetic-formalin solution, was washed and dehydrated in dioxan, and embedded in paraffin. Heidenhain's haematoxylin and a combination of aniline blue and safranine were employed in staining the sections.

## DEVELOPMENT AND BREAKDOWN OF VESSEL END WALLS

*Structure of End Walls in Very Young Vessels.*—In the ontogeny of vessels the mother cells expand and vacuolate before the secondary thickenings develop on their longitudinal walls. Very young mother cells have uniformly thin walls (fig. 1, below). When the cell reaches its mature diameter but still contains a nucleus and cytoplasm, its longitudinal walls are thin, whereas the transverse end walls are characteristically thickened (fig. 1, above). This thickening, which in longitudinal sections of cells appears lenticular in shape, occurs only on that portion of the end wall which is to be removed during maturation of the vessel, whereas the rest of the end wall is thin.

In prepared sections the thickened portion of the end wall usually appears to be limited above and below by two sharp, dark lines enclosing between them a lighter-colored substance. Plates 2, A and 4, A illustrate this condition. They also show the thin marginal region of the end wall.

Not infrequently a line is perceptible in median position in the thickened portion of the end wall. It seems to separate the thickening into two layers (plate 4, K).

<sup>1</sup> Received for publication September 1, 1936.

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<sup>3</sup> See "Literature Cited" at the end of the paper for complete data on citations, which are referred to in the text by author and date of publication.

Although studies on vessels reported here do not suffice for conclusions regarding the nature of the thickened portion of the end wall, the suggestion might be made that the thickening is primary in nature, similar perhaps to the torus thickening in bordered pits. The line dividing the thickening in two halves in some sections might be—using Kerr and Bailey's classification (1934)—the intercellular substance.

Ontogenetically this thickening is not related to the secondary wall that later appears in vessels; and its staining reactions seem to correspond to those of the primary wall. A particularly good contrast between the end wall and the secondary deposits on vessel walls may be obtained

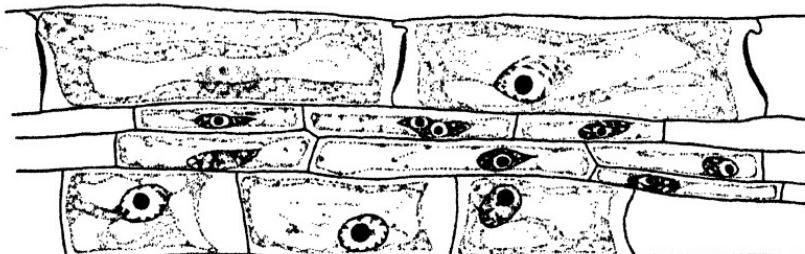


Fig. 1.—Longitudinal section from the primary xylem showing two rows of xylem mother cells and young xylem parenchyma between them. ( $\times 590$ .)

with aniline blue and safranine. The latter is retained by the lignified secondary thickening, whereas a bright blue color is obtained in the primary part of the longitudinal walls and in the entire end wall.

*End Walls During Deposition of Secondary Walls.*—After the end wall has been thickened, the vessel element develops secondary wall layers—spiral, scalariform, or reticulate. To illustrate these later stages of vessel development, spiral elements will be used.

The coils of the spirals are deposited not only upon the longitudinal walls but also upon the thin portion of the end wall. Plate 2, *B* shows this condition in considerable detail. The coils of the spiral seen in optical cross section appear as knobs on the inner surface of the primary walls. Two coils were deposited on each side of the thin portion of the end wall, leaving the lenticular thickening exposed. Thin threads of the parietal cytoplasm appear above and below the end wall. The nucleus of one of the elements is discernible at the left below.

The same section as in plate 2, *B* has been used for the drawing in plate 4, *D*. Two young elements with immature secondary walls are shown in their entirety in plate 3, *A* and *B*. Nuclei occupy central positions in the cells, and the end walls appear thick and dark.

In the first protoxylem vessels no coils are laid over the end wall; and

the thin portion of this wall is, correspondingly, very narrow (plate 4, *A*, *B*, and *C*). It is wider when one or more coils are deposited on the end wall (plate 4, *D*). In some of the later protoxylem or in the metaxylem elements only a comparatively small area of the end wall becomes perforated, in which case the lenticular thickening is of a very limited extent (plate 4, *J*).

Sometimes the two superimposed vessel elements do not begin the secondary-wall development simultaneously, but one lags behind the other (plate 4, *B*).

The end wall is readily discerned in transverse sections of xylem. It obstructs the light no less than parenchyma walls when these are seen in face view. In plate 1 both photographs show, at the right, closed vessels with lumina darkened by the transverse end walls. In both these vessels one coil of secondary thickening is lying over the end wall. Open vessels appear at the left in each photograph. Plate 1, *B* also shows, at the lower right, a young vessel element with a parietal layer of cytoplasm and immature secondary wall. It has been cut away from either of its two end walls.

Plate 4, *L* represents an end wall of an immature vessel. The lenticular thickening seen from above is darker than the thin margin. Faint lines appear to radiate from a barely perceptible ring on the end wall. Although the nature and significance of the radiating lines are not understood, the faint ring is secondary-wall material that is being deposited on the end wall.

Very frequently coarse granules or flakes of disintegrating cytoplasm obscure the visibility of end walls in transverse sections (plate 1, *B*). Sometimes, however, the cytoplasm is removed in cutting, and the end wall is exposed to view (plates 1, *A* and 4, *L*). In such cases the end wall usually shows numerous small dark spots on a lighter background. Sectional views of the end wall seem to indicate that these spots are granules on the surface of the wall (plate 4, *D*).

In many sections the end wall is broken in cutting on the microtome. In such cases the fragments may become tilted (plate 4, *M*).

The cytoplasm of developing vessels manifests a notable behavior. In vessel mother cells it shows considerable shrinkage in the fixed material (fig. 1; and plates 1, *B*; 2, *A*; 4, *A*), but as development progresses it gradually loses this tendency to shrink. In *B* of plate 4 the cytoplasm in the lower element receded only slightly from the knobs of secondary thickening, and its outline shows serrations corresponding to the intervals between the knobs. In 4, *C* the cytoplasm adheres to the longitudinal walls, whereas in 4, *D* it is close to the end wall also. As was mentioned

in the previous paper (Esau, 1936), sieve tubes show a similar decrease in tendency to shrink in progressively older elements.

*Breakdown of End Walls.*—The vessel end wall breaks down when the secondary wall is mature and the protoplast is ready to disappear. The end wall becomes thinner until but faint threads, like cobwebs, occur in its place and then vanish also. The thinning down of the end wall seems to indicate that it is dissolved.

In the early stages of this process the disintegrating cytoplasm, which stains very dark, usually obscures the clearness of the picture. Such a condition is shown in plate 3, C, where a very thin end wall is covered with a heavy layer of coarse disintegrating cytoplasm; F, G, and I in plate 4 show final stages in the breakdown of the end wall.

Portions of mature spiral vessels are shown in plates 2 C, D, and 4, E. The protoplasts and the lenticular part of the end wall have disappeared. Whereas two coils of secondary thickening form the rim between two sections of vessels in plates 2, C and 4, E, one coil forms it in plates 1, A and 2, D.

The primary wall, which is clearly visible between the upper and lower coils of the rim in plates 2, D and 4, E, is the thinner portion of the end wall that surrounded the lenticular thickening. By focusing up and down one can follow this primary wall all around the rim. This situation is indicated in plate 4, H, which shows the same vessel as plate 4, E, but as it appears at a lower focus. This primary wall is seldom discernible between the two adjacent coils, so that the sections of the pairs of coils of the rim seem to be detached from the rest of the wall (plates 2, C and 4, E). The primary wall may also appear very faint between the coils of the spiral on longitudinal walls (plate 4, G, left).

In a series of superimposed vessel elements the end walls disappear not simultaneously but one after the other, the process starting at one end of the vessel and progressing toward the other. Stages from an intact end wall to its complete absence may be found in one section of a vessel in which disintegration of end walls is in progress.

In plate 3, C the other end of the lower element had a thick end wall, while the other end of the upper element appeared as is shown in the photograph in plate 3, D. Here the end wall was absent, but the degenerated cytoplasm was still present. E, F, and G in plate 4 represent a sequence of end walls in elements of the same vessel. The end wall following that shown in G was still intact. One end of the lower vessel element in 4, I was closed; the other end of the upper element was free of end-wall fragments.

## DISCUSSION

These observations on development and perforation of end walls in vessel elements in celery fail to agree with the description given by Priestley and his co-workers (1935) regarding vessel differentiation in several woody species of angiosperms. These authors place the actual perforation of the end wall at a very early stage in the development of the vessel mother cell, namely before secondary wall thickenings are initiated, although "pectin films are often left stretched across the perforated ends of the vessel segments" (Priestley, *et al.* 1935, p. 53). Because of these thin mucilaginous films the protoplasts of individual elements do not mingle.

Still less similarity exists between the behavior of vessels in celery and vessel differentiation described by Scott (1935) in *Ricinus*, where these elements are said to pass through a coenocytic stage before secondary-wall formation. This behavior in *Ricinus* is held by Scott to cause the development of long spirals continuous through many segments.

In celery the spirals are not continuous but show distinct breaks at the ends of each element. This break appears only as a change in the twist in earlier-formed vessels and as a rim, partly obstructing the pore, in vessels differentiating later in the development of a vascular bundle.

Vessel development in celery agrees in general features with that given for *Robinia pseudoacacia* by Eames and MacDaniels (1925, p. 151). These authors, however, show, in their figure 75, C, deposition of secondary wall on the part later to be removed. In the celery the thickening on the end wall appears to be primary and, as was suggested before, similar to the torus thickening in bordered pits.

Eames and MacDaniels (1925, fig. 75, D) also show the nucleus lying adjacent to the wall where dissolution is occurring. In the celery material the nucleus was usually found in median position in young differentiating elements (plate 3, A and B), but nearer one of the longitudinal walls in the more mature highly vacuolated cells (plate 2, B). It was not observed at the end walls.

In view of the contrasting interpretation by different workers of the phenomena involved in vessel differentiation in angiosperms, further studies would be pertinent, particularly if combined with microchemical tests.

### SUMMARY

The primary vessels in celery show intact end walls until the elements are almost mature. These walls show a peculiar lenticular thickening in the region that later becomes the perforation.

Secondary thickenings are formed on longitudinal walls and usually also on the margins of the end wall around the lenticular thickening, leaving the latter exposed.

When the end wall breaks down, the lenticular thickening disappears gradually, as though dissolved. This change occurs after the secondary thickenings have been deposited. The protoplast disintegrates at the same time.

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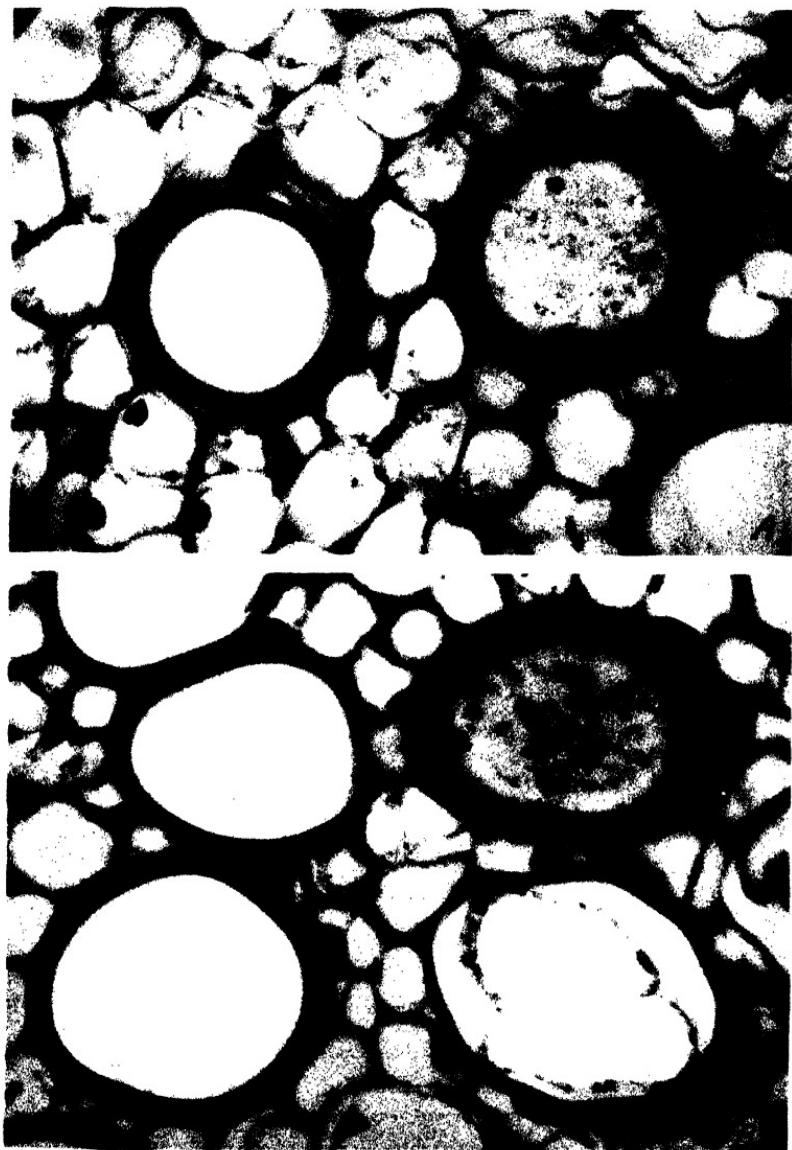


Plate 1.—Transverse sections of xylem showing contrast between immature and mature vessels: *A*, one immature vessel at the right showing intact end wall; *B*, two immature vessels at the right. The one at the upper right shows an intact end wall with flaky degenerating cytoplasm covering it; the other (lower right) has a parietal layer of cytoplasm and immature secondary thickening. ( $\times 833$ .)



Plate 2.—Longitudinal sections of vessels cut through the union of two vessel elements: *A*, lenticular thickening on the end wall, but no spiral thickenings on the longitudinal walls; *B*, end wall still intact and spiral thickenings on longitudinal walls and on the thin portion of the end wall; *C, D*, mature vessels, the end wall having disappeared except for the portion covered by the rim of secondary thickening. ( $\times 833$ .)



Plate 3.—*A, B*, Young vessel elements showing nuclei, thickened end walls, and immature spiral secondary thickenings. *C*, Disintegration of end wall between two vessel elements. *D*, Final stage in end-wall disintegration; the cytoplasm is still evident. (All longitudinal sections,  $\times 833$ .)

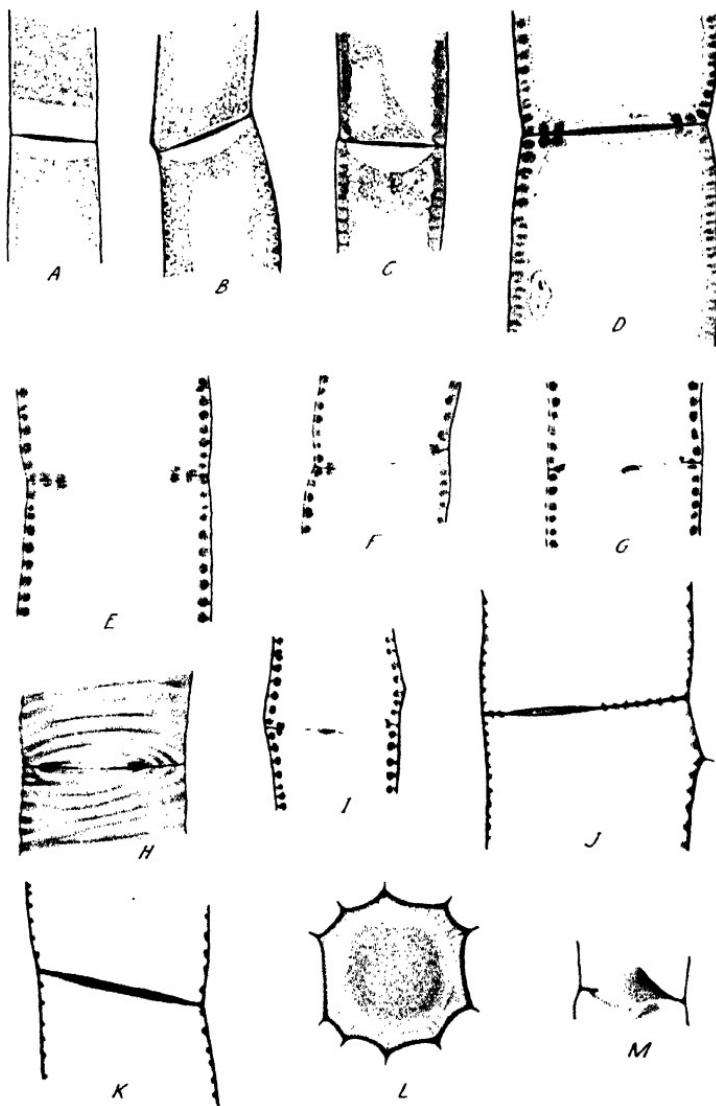


Plate 4.—Development and breakdown of the vessel end wall: *A-D*, show thickened end wall and successive stages of development of spiral secondary thickenings; *E, H*, rim between two mature vessel elements shown at two different focal planes; *F, G, I*, disintegration of the end wall; *J*, end wall of vessel elements that in a mature state communicate through a small perforation; *K*, shows the median line in the thickened end wall; *L*, end wall in face view; *M*, end wall broken in preparation. ( $\times 700$ .)

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## INHERITANCE OF CERTAIN FRUIT AND SEED CHARACTERS IN WATERMELONS<sup>1</sup>

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### INTRODUCTION

WITH THE INCREASED INTEREST being manifested in the development of new varieties of watermelons, *Citrullus vulgaris* (Schrad.), additional information is needed on the mode of inheritance of certain quantitative and qualitative characters. Many of the newer strains resistant to *Fusarium* wilt have resulted from hybridization of parents of diverse foliage, fruit, and seed characters. As experience has demonstrated, it is possible to establish and maintain resistance, but more difficult to purify new strains with respect to certain other characters. The plant breeder should not release resistant strains to the trade until they are relatively homozygous for seed characters, fruit type, fruit-skin color, and are uniformly satisfactory in quality. The mode of inheritance has a definite bearing on the problem.

The watermelon has not been extensively analyzed genetically or cytologically, probably because of the relatively large area of land necessary to mature fruits in sufficient quantity to provide a population for genetic analysis. The crop is somewhat limited, furthermore, by regional adaptation and by the length of the growing season; and it is not particularly high in food value.

Of the many varieties of watermelons known, relatively few are grown extensively. Regional adaptation, market preference, and wilt resistance determine the variety or varieties preferable for a particular district. In the North, where frosts occur early in the fall, quick-maturing varieties are needed. Southern districts favor varieties with a tough (usually thick) rind suited for shipment to the northern markets. Whereas some

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markets demand very large (30 to 35 pounds) fruits, others prefer fruits averaging 20 pounds or less. To meet these demands, varieties have been developed that vary in such characters as wilt resistance; fruit-skin color; rind toughness and thickness; flesh color, texture, and sugar content; seed size; seed-coat color; and fruit size and shape. To facilitate further breeding operations the mode of inheritance is here reported for a number of these characters.

#### REVIEW OF LITERATURE

Watermelon inheritance has not been extensively studied except as it pertains to the development of wilt-resistant varieties. As early as 1911 Orton<sup>(1)</sup> stated that resistance to wilt was inherited as a recessive character, since all  $F_1$  plants became infected with wilt if grown in infested soil. He noted apparent hybrid vigor in the  $F_1$  generation, with apparent dominance of skin striping and of inedible flesh in crosses involving the wilt-susceptible, edible, striped variety, Eden, and a wilt-resistant, inedible variety, Citron. The  $F_2$  was extremely variable in type and quality, with inedibility dominant in a large percentage of the resistant plants. Orton had no opportunity to study the mode of inheritance of fruit characters critically, for his  $F_2$  and backcross populations were grown in wilt-infested soil where susceptible plants died before maturing their fruit. Eight generations of selection for desirable fruit type, quality, and wilt resistance produced the resistant variety Conqueror.

Porter and Melhus<sup>(4)</sup> likewise found that resistance to wilt was inherited as a recessive character; and their experience in selecting for resistance among edible, susceptible varieties indicates that resistance is due to multiple factors, though no detailed study was made. Their data on segregation of fruit type and quality in the  $F_2$  and  $F_3$  of watermelon-citron crosses do not indicate the mode of inheritance, because these populations were grown in infested soil where susceptible plants died before or soon after forming blossoms. The results agree with Orton's in that inedibility is dominant; but they further show that the white, hard flesh of the Citron is dominant to the red, relatively soft flesh of edible commercial varieties such as Kleckley Sweet and Halbert Honey. In the  $F_2$  and  $F_3$  generations the flesh color was white, yellow, pink, or red. In certain  $F_3$  fruits the flesh was red or yellow near the seeds but otherwise white. The green, red, and purple seed-coat color of the various Citron strains was dominant to the white seed coat of Kleckley Sweet and Halbert Honey.

\* Superscript numbers in parentheses refer to "Literature Cited" at the end of this paper.

Rosa,<sup>(6)</sup> in 1928, showed that in crosses between monoecious and andromonoecious varieties of watermelons, the monoecious condition depended upon a single dominant factor. He secured close approximation



Fig. 1.—Left, Golden Honey (yellow flesh, white seeds, and striped skin); right, Angeleno (red flesh, black seeds, and green skin); center, the F<sub>1</sub> hybrid (cross 17) of these parents (red flesh, black seeds, and very faintly striped skin).

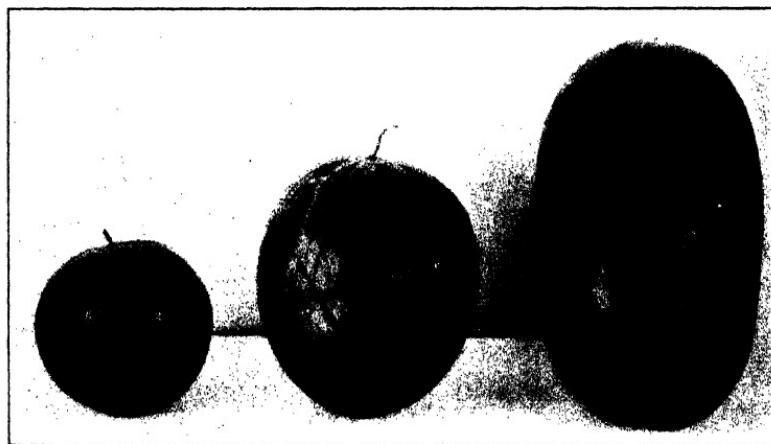


Fig. 2.—Left, Baby Delight (light-tan seed coat); right, California Klondike (black seed coat); center, cross 84, the F<sub>1</sub> of these two parents.

to a 3:1 ratio in the F<sub>2</sub>, and 1:1 in the backcross of the F<sub>1</sub> to the recessive parental variety.

Kanda<sup>(7)</sup> investigated the genetic constitution of several varieties of watermelons producing white, reddish, brown, yellowish-white, reddish-orange, and yellowish-green seed coats as well as certain varieties pro-

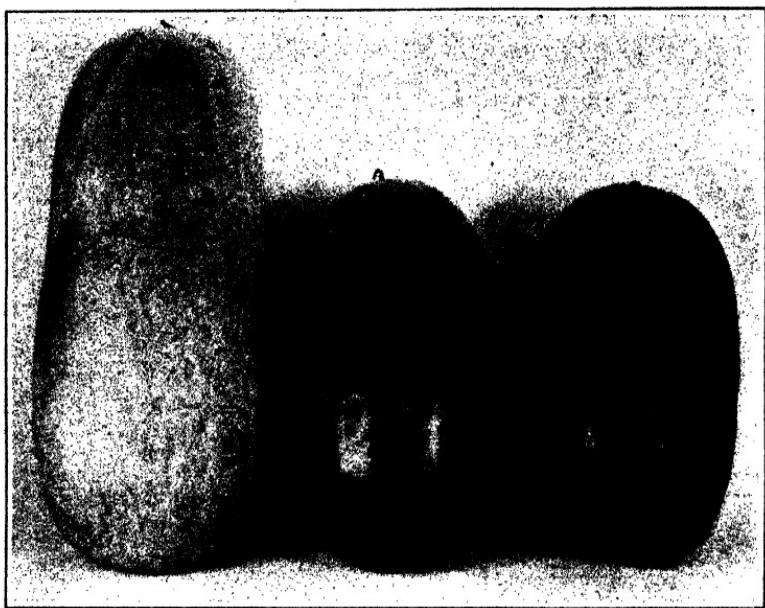


Fig. 3.—Thurmond Grey (left), California Klondike (right), and the F<sub>1</sub> fruit of this cross (center). The skin of the hybrid melon is much lighter green than that of the Klondike and lacks the markings characteristic of Thurmond Grey.

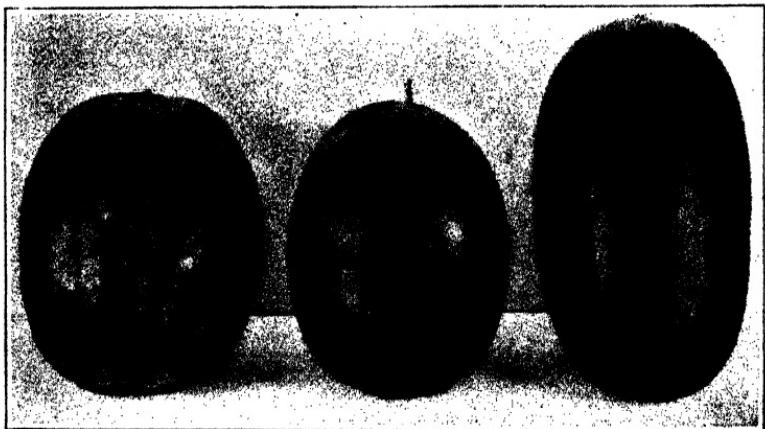


Fig. 4.—Left, Golden Honey (yellow flesh, white seeds, tough rind, and striped skin); right, California Klondike (red flesh, black seeds, tender rind, and dark-green skin); center, the F<sub>1</sub> hybrid (cross 85) of these parents (red flesh; black seeds; light-green, faintly striped skin; and tough rind).

ducing mottled seed coats. He proposed seven pairs of independently inherited factors for seed-coat color.

McKay<sup>(9)</sup> has recently shown that red seed-coat color is recessive to both tan and green in preserving and stock Citrons and that 3:1 ratios were observed in the F<sub>2</sub>.

#### DESCRIPTION OF VARIETIES USED AS PARENTS

Only one yellow-fleshed variety, Golden Honey, was used. Before being employed as a parental type it was selfed for three generations, and extensive tests have shown that the inbred strains are pure for yellow flesh color as well as for white-seededness. The other varieties used—Angeleno, California Klondike, Thurmond Grey, Baby Delight, Chilean, Snowball, and Pride of Muscatine—always produce red flesh. Angeleno and California Klondike are black-seeded; Thurmond Grey is dark tan; Baby Delight is light tan; Chilean, Snowball, and Pride of Muscatine have white seeds.

The fruit-skin colors of the varieties used are as follows: Angeleno (fig. 1), very dark green; California Klondike (fig. 2), dark green; Thurmond Grey (fig. 3), yellowish green; Snowball, yellowish white; and Golden Honey (fig. 4), striped.

The rind of Golden Honey and Thurmond Grey is tough and thick, whereas the rind of California Klondike is tender and thin.

#### METHODS

The varieties used were inbred for several (three to eight) generations, and subsequent tests showed them to be homozygous for the characters studied.

All crosses, backcrosses, and self-pollinations were made by hand, according to the technique described in an earlier paper.<sup>(10)</sup> In the crosses involving the andromonoecious varieties Angeleno, Baby Delight, and Chilean, emasculation was necessary only in cross 21 (table 1), involving the two latter varieties. Otherwise the andromonoecious varieties were used as pollen parents. In certain backcrosses, emasculation was performed approximately 48 hours before anthesis of the perfect flower, as careful tests showed that pollen was not shed at this time. In selfing the F<sub>1</sub> of crosses 21 and 27 (table 1), in which both parents are andromonoecious, a camel's-hair brush was used instead of the male flower, as previously described.<sup>(10)</sup> Alcohol was used for sterilizing the pollinating brushes.

Seed was planted at the rate of approximately ten per hill. The hills were thinned to either one or two plants. At maturity two fruits were picked from each plant, cut, and classified for the characters concerned.

PHENOTYPE OF THE F<sub>1</sub>

The F<sub>1</sub> characteristics of the hybrids discussed herein are indicated in table 1. In every case red flesh was dominant over yellow. Black and tan seed-coat colors were each dominant over white, and black over tan.

TABLE 1  
F<sub>1</sub> CHARACTERISTICS OF CERTAIN WATERMELON HYBRIDS

Cross No.	Parents and characters		F <sub>1</sub> characteristics
17	Angeleno Black seed coat Red flesh Andromonoecious	Golden Honey White seed coat Yellow flesh Monoecious	Black seed coat Red flesh Monoecious
21	Baby Delight Light-tan seed coat	White Seeded Chilean White seed coat	Light-tan seed coat
25	Klondike Green fruit skin	Snowball Yellowish-white fruit skin	Green fruit skin
27	Angeleno Black seed coat Green fruit skin	Snowball White seed coat Yellowish-white fruit skin	Black seed coat Green fruit skin
84	California Klondike Black seed coat Oblong fruit Monoecious	Baby Delight Light-tan seed coat Round fruit Andromonoecious	Black seed coat Intermediate fruit shape Monoecious
85	California Klondike Black seed coat Red flesh Green fruit skin Tender rind	Golden Honey White seed coat Yellow flesh Striped fruit skin Tough rind	Black seed coat Red flesh Intermediate fruit-skin color Tough rind
86	California Klondike Black seed coat Green fruit skin Tender rind	Thurmond Grey Dark-tan seed coat Yellowish-green fruit skin Tough rind	Black seed coat Intermediate skin color Tough rind
89	California Klondike Black seed coat	Pride of Muscatine White seed coat	Black seed coat

In other crosses for which no F<sub>2</sub> or backcross data are available, F<sub>1</sub> manifestations of seed-coat color were as follows:

- Klondike (black) × Iowa Belle (white) = black
- Citron (red) × Snowball (white) = red
- Angeleno (black) × Citron (green) = black
- Citron (red) × Citron (green) = green

Green fruit skin was dominant over yellowish white but incompletely dominant over striped and over yellowish green. In one cross the F<sub>1</sub> of oblong × round fruit was intermediate. Tough rind was dominant over tender, and monoecism was dominant over andromonoecism.

### SEGREGATIONS

**Flesh Color.**—In the  $F_2$  of cross 17, involving Angeleno and Golden Honey (fig. 1), 154 plants were grown. The observed ratio of red to yellow flesh was very close to the calculated 3:1 segregation, with a  $\frac{D}{PE}$  ratio of 1.79 (table 2). In backcrosses 39 and 42 involving cross 17  $\times$  Golden

TABLE 2

**SEGREGATION OF FLESH COLOR IN THE  $F_2$  GENERATION AND IN THE BACKCROSS TO THE RECESSIVE PARENT**

Cross (C) or backcross (BC)	Total plants	Parents and characters	Observed segregation	Expected segregation	$\frac{D}{PE}$
C'17-1	154	Angeleno (red) Golden Honey (yellow)	122 red 32 yellow	115.5 red - 38.5 yellow	1.79*
C'34-1	307	Angeleno (red) Golden Honey (yellow)	244 red 63 yellow	230.25 red 76.75 yellow	2.68*
C'85-1	301	California Klondike (red) Golden Honey (yellow)	236 red 65 yellow	225.75 red 75.25 yellow	2.02*
BC'39 and BC'42	377	C'17 (red) Golden Honey (yellow)	204 red 173 yellow	188.5 red 188.5 yellow	2.36†
BC'40 and BC'41	434	C'85 (red) Golden Honey (yellow)	223 red 211 yellow	217 red 217 yellow	0.85†

\* Involving a 3:1 ratio.

† Involving a 1:1 ratio.

Honey (recessive for flesh color) the  $\frac{D}{PE}$  for the expected 1:1 ratio was 2.36. In cross 34, involving the same varieties as cross 17 but made between different plants, the  $\frac{D}{PE}$  for the expected 3:1 ratio in the  $F_2$  population of 307 plants was 2.68.

Cross 85 (table 2) was made between California Klondike (red flesh) and Golden Honey (yellow flesh). In the  $F_2$  population of 301 plants, the  $\frac{D}{PE}$  for the expected 3:1 ratio was 2.02. The  $\frac{D}{PE}$  for the expected 1:1 backcross ratio in BC'40 and BC'41 was 0.85.

Thus in the  $F_2$  generation of three crosses and in four backcrosses, the segregation shows a single-factor difference between red and yellow flesh color. The symbol *R* is suggested for red, and *r* for yellow flesh color.

**Seed-Coat Color.**—Crosses 17 and 34, previously mentioned, involved black (Angeleno) and white (Golden Honey) seed-coat colors. The seeds

of Angeleno are intensely colored, almost coal black, in contrast to other varieties that may produce sooty-black or dull-black seed coats. Of 461

$F_2$  plants (table 3), the  $\frac{D}{PE}$  for the expected 3:1 ratio with black seed-

TABLE 3

SEGREGATION OF SEED-COAT COLORS IN THE  $F_2$  GENERATION AND IN THE BACKCROSS TO THE RECESSIVE PARENT

Cross (C) or backcross (BC)	Total plants	Parents and characters	Observed segregation	Expected segregation	$\frac{D}{PE}$
C17-1 and C 34-1	461	Angeleno (black) Golden Honey (white)	355 black 106 white	345.75 black 115.25 white	1.47*
C21-1	198	Baby Delight (light tan) Chilean (white)	155 light tan 43 white	148.5 light tan 49.5 white	1.58*
C27-1	217	Angeleno (black) Snowball (white)	159 black 58 white	162.75 black 54.25 white	0.87*
C84-1	387	California Klondike (black) Baby Delight (light tan)	276 black 111 light tan	290.25 black 96.75 light tan	2.48*
C86-1	429	California Klondike (black) Thurmond Grey (dark tan)	328 black 101 dark tan	321.75 black 107.25 dark tan	1.03*
C89-1	108	California Klondike (black) Pride of Muscatine (white)	88 black 20 white	81 black 27 white	2.30*
BC39 and BC42	377	C17 (black) Golden Honey (white)	196 black 181 white	188.5 black 188.5 white	1.14†
BC36	237	C84 (black) Baby Delight (light tan)	120 black 117 light tan	118.5 black 118.5 light tan	0.29†
BC38 and BC43	300	C86 (black) Thurmond Grey (dark tan)	166 black 134 dark tan	150 black 150 dark tan	2.74†

\* Involving a 3:1 ratio.

† Involving a 1:1 ratio.

coat color dominant was 1.47, an excellent fit for this calculated segregation. When the  $F_1$  was backcrossed (BC39 and BC42) to Golden Honey, the recessive parent, the  $\frac{D}{PE}$  for the expected 1:1 ratio was 1.14 among a population of 377 plants. Seed-coat color of cross 17 and of the parental varieties of cross 17 is shown in figure 5.

Baby Delight (light tan) was crossed with Chilean (white) to produce cross 21. A 3:1 ratio was secured in the  $F_2$ , with a  $\frac{D}{PE}$  value of 1.58 involving 198 plants (table 3). Seed-coat color of the two parents of the  $F_1$  is shown in figure 6.



Fig. 5.—Seeds of Golden Honey (left), of Angeleno (right), and (center) the  $F_1$  of these two varieties (cross 17).

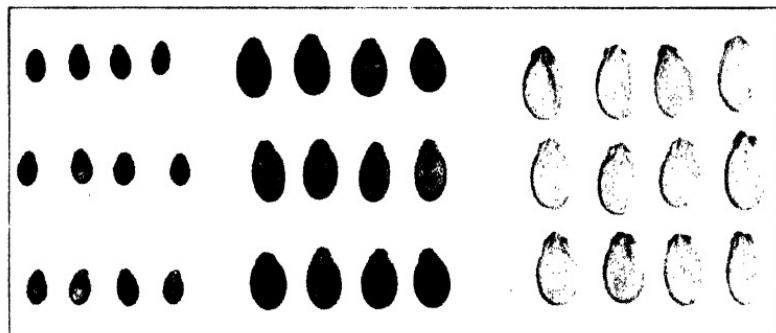


Fig. 6.—Seeds of Baby Delight (left), of Chilean (right), and (center) the  $F_1$  of these two varieties (cross 21).

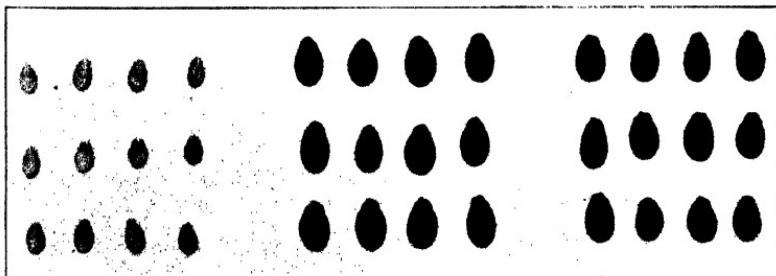


Fig. 7.—Seeds of Baby Delight (left), of California Klondike (right), and (center) the  $F_1$  of these two varieties (cross 84).

In the  $F_2$  generation of cross 27 (table 3), involving Angeleno (black) and Snowball (white), the  $\frac{D}{PE}$  value for a 3:1 ratio was 0.87 when the seeds from 217 plants were examined.

In cross 84 (fig. 7) California Klondike (black) was used as one parent with Baby Delight (light tan) as the other. In the  $F_2$  of this cross, examination of the seeds of 387 plants showed a 3:1 ratio of black to tan with a  $\frac{D}{PE}$  value of 2.48 (table 3). In backcross 36, involving cross 84  $\times$  Baby Delight (the recessive parent), among 237 plants the  $\frac{D}{PE}$  for the expected 1:1 ratio was only 0.29. A single-factor difference was involved.

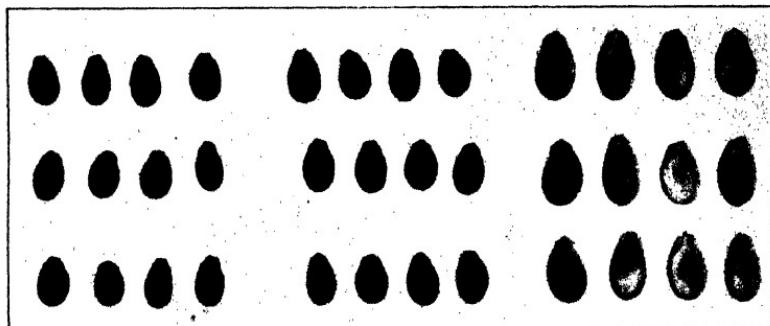


Fig. 8.—Seeds of California Klondike (left), of Thurmond Grey (right), and (center) the  $F_1$  of these two varieties (cross 86).

California Klondike (black) was also crossed with Thurmond Grey (dark tan) to produce cross 86. Among 429  $F_2$  plants, the  $\frac{D}{PE}$  (table 3) for the expected 3:1 ratio was 1.03, with black dominant. Among 300 plants of backcrosses 38 and 43 involving cross 86  $\times$  Thurmond Grey (the recessive parent) the  $\frac{D}{PE}$  value was 2.74. Seed-coat color of the parental varieties and cross 86 is shown in figure 8.

In cross 89, involving California Klondike (black)  $\times$  Pride of Muscatine (white), a segregation for a 3:1 ratio was secured among 108  $F_2$  plants, with a  $\frac{D}{PE}$  value of 2.30 and with black completely dominant.

In every cross involving solid-colored  $\times$  white seeds, the  $F_2$  and back-cross data show that a single pair of factors controls the segregation, with the darker-colored seed possessing the dominant factor. Black ( $B$ ) is dominant to both white ( $b$ ) and tan ( $b^t$ ), and tan is dominant to white.

The possibility still remains that on the basis of two pairs of allelomorphs in which the dominant factors have a complementary interaction, white (*aabb*) when crossed with a tan of the constitution *aaBB* would also give a 3:1 ratio of tan and white. The data are not sufficiently critical, as far as these crosses went, to determine whether or not two pairs of factors or multiple allelomorphs were involved.

**Fruit-Skin Color.**—The inheritance of fruit-skin color was studied in four crosses. Cross 25 involved California Klondike (dark-green skin) and Snowball (yellowish-white skin). The latter variety is not used commercially. The skin of the  $F_1$  fruit is dark green, identical with California Klondike. Among 132  $F_2$  plants the deviation (table 4) from an expected 3:1 ratio was  $4.0 \pm 3.36$ , with a  $\frac{D}{PE}$  value of 1.19, indicating that a single factor controlled fruit-skin color in the cross. No backcross data are as yet available.

Cross 27 involved Angelino (very dark-green skin) and Snowball. The  $F_1$  fruit was identical in color with Angelino; dominance was complete. The deviation in  $F_2$ , involving 216 plants, was  $11.0 \pm 4.29$ , with a  $\frac{D}{PE}$  value of 2.56 (table 4), again indicating a single-factor difference with dark green dominant.

The data on the  $F_2$  generation of crosses 25 and 27 do not definitely prove that a single-factor difference is involved, because no backcross or  $F_3$  populations have yet been grown. The data are presented in contrast to both backcross and  $F_2$  data from cross 86, involving California Klondike (green skin) and Thurmond Grey (yellowish-green skin).

The  $F_1$  fruit skin of cross 86 was intermediate in color between the two parents (fig. 3)—lighter green than California Klondike but darker green than Thurmond Grey. Apparently it is a case of incomplete dominance, in direct contrast to the  $F_1$  fruits of crosses 25 and 27, where dominance of green skin apparently was complete.

The  $F_1$  plants of cross 86 were backcrossed to both Thurmond Grey (BC38 and BC43) and to California Klondike (BC35). These backcross populations as well as the  $F_2$  manifested segregations typical of incomplete dominance involving a single pair of factors (table 4).

According to the data on 429 plants of the  $F_2$  generation, the observed segregation among the three skin-color classes was very close to the expected 1:2:1 ratio; the chi squared ( $\chi^2$ ) value was 4.82, and the odds were 10.01:1 against getting a greater deviation than the one observed.

Among 117 plants of backcross 35, the deviation from an expected 1:1 ratio of dark-green and light-green skin (table 4) was  $3.5 \pm 3.65$ , giving

TABLE 4  
SEGREGATION OF FRUIT-SKIN COLORS IN THE F<sub>2</sub> GENERATION AND IN THE BACKCROSS TO THE RECESSIVE PARENT

Cross or backcross	Total plants	Parents and characters	Observed segregation	Expected segregation	D — PE	$\chi^2$	Odds
C25-1	132	California Klondike (dark green) Snowball (yellowish white)	95 dark green 37 yellowish white	99 dark green 33 yellowish white	1.19*	....	.....
C27-1	216	Angeleno dark green Snowball (yellowish white)	173 dark green 43 yellowish white	162 dark green 54 yellowish white	2.56*	....	.....
C85-1	301	California Klondike (dark green) Golden Honey (striped)	85 dark green 142 intermediate 74 striped	76.25 dark green 150.50 intermediate 75.25 striped	....	1.76†	1.48:1
C86-1	429	California Klondike (dark green) Thurmond Grey (yellowish green)	106 dark green 201 intermediate 122 yellowish green	102.2 dark green 214.5 intermediate 102.2 yellowish green	....	4.82†	10.01:1
BC35	117	C86 (intermediate) California Klondike (dark green)	55 dark green 62 intermediate	58.5 dark green 58.5 intermediate	0.96‡	....	.....
BC38 and BC43	300	C86 (intermediate) Thurmond Grey (yellowish green)	148 intermediate 152 yellowish green	150 intermediate 150 yellowish green	0.34‡	....	.....

\* Involving a 3:1 ratio.

† Involving a 1:2:1 ratio.

‡ Involving a 1:1 ratio.

a  $\frac{D}{PE}$  value of 0.96. Furthermore, among 300 plants the deviation from an expected 1:1 ratio of light-green and yellowish-green skin in back-crosses 38 and 43 was only  $2.0 \pm 5.84$ , giving a  $\frac{D}{PE}$  value of 0.34.

Though the fruit-skin color of Angeleno is somewhat darker green than that of California Klondike, there is insufficient contrast either to determine the  $F_1$  phenotype accurately or to classify the  $F_2$  generation resulting from a cross of these two varieties. Similar difficulty would probably be encountered in crosses of Thurmond Grey and Snowball. Crosses involving Snowball and either Angeleno or California Klondike manifest complete dominance of dark-green skin color. Thus the dark-green skin color of California Klondike appears completely dominant to the yellowish-white skin of Snowball but incompletely dominant to the yellowish-green of Thurmond Grey (fig. 3).

The  $F_1$  fruits of California Klondike  $\times$  Golden Honey (cross 85) manifested a very faint skin stripe, but were distinct from both parents (fig. 4). With incomplete dominance and with a single factor governing segregation, a 1:2:1 ratio would be expected in the  $F_2$ . The data in table 4 indicate this segregation, showing a  $\chi^2$  value of 1.76 and odds of 1.48 to 1 against getting a greater deviation than the one observed. Further studies of the inheritance of skin striping involving other varieties are in progress.

**Rind Toughness.**—Satisfactory shipping quality in watermelons is largely dependent upon a tough, but not necessarily thick, fruit rind. The fact that relatively few thin-rinded varieties are extensively used for long-distance shipment probably means that a thin rind is usually tender. Although this is true of the No. 1 strain of California Klondike<sup>(6)</sup>, the No. 8 strain is characterized by a thin but very tough rind. It is used in Imperial Valley for Canadian and other long-distance shipments. The rind of California Klondike No. 1, although equal in thickness to that of No. 8, is very tender; fruits harvested early in the morning after a cool night must be carefully handled to prevent cracking. This "explosiveness" of the fruit may be due to the thin, tender rind, to the high turgidity of the flesh, or to both. The rind of Thurmond Grey is both thick and tough, and when fruits are cut lengthwise they seldom burst open. The rind seems more woody than that of California Klondike, and the flesh less turgid. This variety is distinctly "nonexplosive." These characteristics, which manifest themselves under the arid condition in California, might conceivably vary, however, under more humid climatic conditions.

Cross 86, previously discussed, involved Thurmond Grey  $\times$  California Klondike. The  $F_1$  fruits are characterized by a tough, woody rind similar to Thurmond Grey but intermediate in thickness between the parents. Toughness, however, is clearly dominant. The relative explosiveness of  $F_2$  fruits was determined by cutting the fruit lengthwise. Admittedly this test is rather crude; but all California Klondike No. 1 fruits were found to be explosive and all Thurmond Grey fruits distinctly nonexplosive when so treated. In the  $F_2$  generation, involving 429 fruits, the  $\frac{D}{PE}$  for the expected 3:1 ratio was 1.44, indicating that tough rind is completely dominant over its allelomorph, tender rind. The symbol *T* is suggested for tough (nonexplosive) rind, and *t* for tender (explosive) rind.

TABLE 5  
SEGREGATION OF RIND TOUGHNESS IN THE  $F_2$  GENERATION

Cross No.	Total plants	Parents and characters	Observed segregation	Expected segregation	$\frac{D}{PE}$
C85-1	152	California Klondike (tender) Golden Honey (tough)	116 tough 36 tender	114 tough 38 tender	0.58
C86-1	429	California Klondike (tender) Thurmond Grey (tough)	313 tough 116 tender	321.75 tough 107.25 tender	1.44

Similar results were secured with cross 85, involving California Klondike  $\times$  Golden Honey. The latter variety has a tough rind. Among 152  $F_2$  plants the  $\frac{D}{PE}$  value for an expected 3:1 ratio was 0.58 (table 5), again indicating complete dominance of the tough rind. Neither  $F_2$  nor backcross data are available.

#### TESTS FOR INDEPENDENT ASSORTMENT

*Flesh Color with Seed-Coat Color.*—Extensive variation in seed-coat color occurs among red-fleshed varieties. These variations extend from pure white through various shades of tan and brown to coal black. In addition to these solid colors, many types of mottling, variegation, and other markings are characteristic of certain varieties. In this paper, attention is directed only to varieties whose seeds have a solid coat color. In all yellow-fleshed varieties with which the writer is familiar, the seed coat is white. Is there close linkage of yellow flesh and white seed-coat color, or could a yellow-fleshed, black-seeded strain be isolated and purified? If the two pairs of factors are independent, the  $F_2$  population should segregate on a 9:3:3:1 basis.

TABLE 6  
TESTS FOR INDEPENDENT ASSORTMENT OF THE FACTORS GOVERNING INHERITANCE OF SEED-COAT COLOR,  
FRUIT-SKIN COLOR, AND FLESH COLOR

Cross or backcross	Total plants	Parents and characters	Phenotype	Observed segregation	Expected segregation	$\chi^2$	Odds
CH7-1	154	Angeleno Red flesh Black seed coat	Golden Honey Yellow flesh White seed coat	Red flesh, black seed coat Red flesh, white seed coat Yellow flesh, black seed coat Yellow flesh, white seed coat	91 35 25 3	86.6 28.9 28.9 9.6	9.67:1*
BC39 and BC42	377	Cross 17 Red flesh Black seed coat	Golden Honey Yellow flesh White seed coat	Red flesh, black seed coat Red flesh, white seed coat Yellow flesh, black seed coat Yellow flesh, white seed coat	103 101 93 80	94.25 94.25 94.25 94.25	5.09:1†
CS6-1	301	California Klondike Red flesh Dark-green skin	Golden Honey Yellow flesh Striped skin	Dark-green skin, red flesh Dark-green skin, yellow flesh Intermediate skin, red flesh Intermediate skin, yellow flesh Striped skin, red flesh Striped skin, yellow flesh	70 15 108 34 58 16	56.4 18.8 113.0 37.6 56.4 18.8	
BC40 and BC41	434	Cross 85 Red flesh Intermediate skin	Golden Honey Yellow flesh Striped skin	Intermediate skin, red flesh Intermediate skin, yellow flesh Striped skin, red flesh Striped skin, yellow flesh	105 103 118 108	108.5 108.5 108.5 108.5	1.22 0.33:1‡

\* Involving a 9:3:3:1 ratio.

† Involving a 1:1:1:1 ratio.

‡ Involving a 3:1:6:2:3:1 ratio.

The data on 154 plants of the  $F_2$  of cross 17, where Angeleno (red, black) was crossed with Golden Honey (yellow, white), show that the observed segregation among the four classes was very close to a 9:3:3:1 ratio; the  $\chi^2$  value was 6.56, and the odds were 9.67:1 against getting a greater deviation than the one observed (table 6). These  $F_2$  data indicate that in this dihybrid the assortment of the factors governing flesh and seed-coat color is independent; they give no indication of linkage.

This dihybrid has not been carried into the  $F_3$  generation; but the  $F_1$  of cross 17 has been backcrossed to Golden Honey, recessive for both flesh and seed-coat color. Since these characters are due to single-factor differences when considered separately, if they are inherited independently the progeny of the backcross should segregate evenly into four classes—red black, red white, yellow black, and yellow white. The data in table 6 for backcrosses 39 and 42, involving cross 17  $\times$  Golden Honey, show that the observed segregation in the backcross population of 377 plants fits the 1:1:1:1 ratio with odds of 5.09:1 of getting a greater deviation than the one observed. These backcross data again indicate independent assortment of the factors governing flesh and seed-coat color in this cross.

*Flesh Color with Skin Color.*—Cross 85 was made between California Klondike (red flesh, dark-green skin) and Golden Honey (yellow flesh, striped skin). Red flesh was completely dominant (table 2) and the  $F_1$  fruit-skin color was intermediate between that of the two parents, with a 1:2:1 ratio in the  $F_2$  (table 4). Thus a ratio of 3:1:6:2:3:1 should be observed in the  $F_2$ .

In like manner in the backcross of the  $F_1$  to Golden Honey there should be equal distribution into four classes, with no fruits manifesting the dark-green skin characteristic of California Klondike.

The  $F_2$  data derived from cross 85 (table 6) indicate independent assortment of the factors governing flesh color and fruit-skin color. The  $\chi^2$  value is 5.05, with odds of 1.41:1 against getting a greater deviation than the one observed.

Backcross data likewise fail to indicate linkage of flesh color and fruit-skin color. All fruits of BC40 and BC41 manifested either intermediate or striped skin, and there was equal distribution into the four classes indicated. The  $\chi^2$  value was 1.22, and the odds were 0.33:1.

*Fruit-Skin Color with Rind Toughness.*—Since incomplete dominance of fruit-skin color and complete dominance of rind toughness had been found in cross 86, data were available to test the possibility of linkage of the factors governing these characters.

According to the data in table 7, the observed segregation in  $F_2$  of C86

TABLE 7  
TESTS FOR INDEPENDENT ASSORTMENT OF THE FACTORS GOVERNING INHERITANCE OF FRUIT-SKIN COLOR,  
RIND TOUGHNESS, AND SEED-COAT COLOR

Cross No.	Total plants	Parents and characters	Phenotype	Observed segregation	Expected segregation	$\chi^2$	Odds
C85-1	152	California Klondike Dark-green skin Tender rind	Golden Honey Striped skin Tough rind	Dark-green skin, tough rind Dark-green skin, tender rind Intermediate skin, tough rind Intermediate skin, tender rind Striped skin, tough rind Striped skin, tender rind	26 7 60 21 28 10	28.5 9.5 57.0 19.0 28.5 9.5	0.19:1
C86-1	429	California Klondike Dark-green skin Tender rind	Thurmond Grey Yellowish-green skin Tough rind	Dark-green skin, tough rind Dark-green skin, tender rind Intermediate skin, tough rind Intermediate skin, tender rind Yellowish-green skin, tough rind Yellowish-green skin, tender rind	73 33 145 56 95 27	80.4 26.8 161.0 53.6 80.4 26.8	2.74:1
C86-1	429	California Klondike Black seed coat Tender rind	Thurmond Grey Dark-tan seed coat Tough rind	Black seed coat, tough rind Black seed coat, tender rind Dark-tan seed coat, tough rind Dark-tan seed coat, tender rind	238 90 75 26	241.4 80.4 80.4 26.8	1.58†
C85-1	152	California Klondike Red flesh Tender rind	Golden Honey Yellow flesh Tough rind	Red flesh, tough rind Red flesh, tender rind Yellow flesh, tough rind Yellow flesh, tender rind	89 29 27 7	85.5 28.5 28.5 9.5	0.88†

\* Involving a 3:1:6:2:3:1 ratio.

† Involving a 9:3:3:1 ratio.

was very close to the expected 3:1:6:2:3:1 ratio, indicating independent assortment of the factors governing fruit-skin color and rind toughness, with no indication that the rind toughness was linked with the yellowish-green fruit-skin color in Thurmond Grey. The  $\chi^2$  value was 6.46, with odds of 2.74:1 against getting a greater deviation than the one observed. The number of plants involved was 429.

Similar data for cross 85 (California Klondike  $\times$  Golden Honey), involving skin color and rind toughness, appear in table 7. Among 152 F<sub>2</sub> plants the observed segregation was very close to the expected, with a  $\chi^2$  value of 1.25 and with odds of only 0.19:1.

The available F<sub>2</sub> data make it probable that linkage of the factors controlling rind toughness and fruit-skin color does not exist. Backcross data are not available to substantiate the evidence.

*Rind Toughness with Seed-Coat Color.*—The data covering cross 86 (California Klondike  $\times$  Thurmond Grey) were further analyzed for possible linkage of rind toughness with seed-coat color. Each of these characters was due to a single-factor difference, with complete dominance of tough rind and of black seed coat.

As the data in table 7 show, the observed F<sub>2</sub> segregation approximated the expected for a 9:3:3:1 ratio, with a  $\chi^2$  value of 1.58 and with odds of 0.44:1 against getting a greater deviation than the one observed. Apparently, though neither backcross nor F<sub>3</sub> data are available, the factors that govern rind toughness and seed-coat color are not linked but are independently inherited.

*Rind Toughness with Flesh Color.*—Since in cross 85 (tables 2 and 5) rind toughness as well as flesh color is determined by a single-factor difference, the F<sub>2</sub> population of this cross should segregate on a 9:3:3:1 basis. The data in table 7 indicate such segregation with a  $\chi^2$  value of 0.88 and odds of 0.21:1, indicating that linkage did not occur.

*Fruit-Skin Color with Seed-Coat Color.*—The relation of seed-coat color to fruit-skin color was studied in cross 86 (California Klondike  $\times$  Thurmond Grey) and in backcrosses 38 and 43 involving cross 86 and Thurmond Grey, which is the recessive parent for seed-coat color and is incompletely dominant for fruit-skin color. Data on the F<sub>2</sub> of cross 86 and backcrosses 38 and 43 appear in table 8.

The data on 429 plants of the F<sub>2</sub> generation of cross 86 show that the observed segregation among the six classes was very close to a 3:1:6:2:3:1 ratio; the  $\chi^2$  value was 5.10, and the odds were 1.45 to 1 against getting a greater deviation than the one observed (table 8).

Substantiating the F<sub>2</sub> data, some data for the two characters in backcross 38 and 43 also appear in table 8. Since F<sub>1</sub> fruit-skin color was

TABLE 8  
TESTS FOR INDEPENDENT ASSORTMENT OF THE FACTORS GOVERNING INHERITANCE OF FRUIT-SKIN COLOR AND SEED-COAT COLOR

Cross or backcross	Total plants	Parents and characters		Phenotype	Observed segregation	Expected segregation	$\chi^2$	Odds
		Parents	Characters					
C36-1	429	California Klondike	Thurmond Grey	Dark-green skin, black seed coat	82	80.4		
		Dark-green skin	Yellowish-green skin	Dark-green skin, dark-tan seed coat	24	26.8		
		Black seed coat	Dark-tan seed coat	Intermediate skin, black seed coat	158	161.0	5.10*	1.45:1
				Intermediate skin, dark-tan seed coat	43	53.6		
				Yellowish-green skin, black seed coat	88	80.4		
				Yellowish-green skin, dark-tan seed coat	34	26.8		
BC38 and BC43	300	C36	Thurmond Grey	Intermediate skin, black seed coat	78	75		
		Intermediate skin	Yellowish-green skin	Intermediate skin, dark-tan seed coat	69	73	2.58†	1.15:1
		Black seed coat	Dark-tan seed coat	Yellowish-green skin, black seed coat	85	75		
				Yellowish-green skin, dark-tan seed coat	65	75		

\* Involving a 3:1:3:2:3:1 ratio.

† Involving a 1:1:1:1 ratio.

intermediate, when the  $F_1$  is crossed back on Thurmond Grey the resulting population should segregate evenly into four groups.

The data on 300 plants of backcrosses 38 and 43 (table 8) show practically equal segregation into four classes. The  $\chi^2$  value is 2.58, and the odds are 1.15:1.

The data for cross 86 and backcrosses 38 and 43 thus indicate independent assortment of the factors governing fruit-skin color and seed-coat color. There is no suggestion of linkage relations.

### SUMMARY

In the crosses studied, red flesh color (*R*) is dominant over yellow (*r*); and segregation in the  $F_2$  generation and backcrosses indicates a single-factor difference.

In crosses involving black and white seeds, black (*B*) is dominant over white (*b*). Here, again, single-factor differences occur, as shown by  $F_2$  and backcross segregants. Likewise, in crosses involving black (*B*) and tan (*b'*), the dominant black color is due to a single factor. Tan (*b'*) was dominant over white (*b*).

The yellowish-white skin color characteristic of the variety Snowball is recessive to the dark-green skin color of Angeleno and California Klondike and is governed by a single-factor difference.

In cross 86 (California Klondike  $\times$  Thurmond Grey), involving dark-green  $\times$  yellowish-green skin, the  $F_1$  fruits manifested incomplete dominance, favoring the dark-green skin of California Klondike; and 1:2:1 segregation occurred in the  $F_2$ . Backcross 35 (cross 86  $\times$  California Klondike) segregated evenly into dark-green and intermediate groups. Backcrosses 38 and 43 (cross 86  $\times$  Thurmond Grey) segregated evenly into intermediate and yellowish-green groups. A single-factor difference apparently governed segregation.

Skin stripedness of Golden Honey was incompletely dominant to dark-green skin of California Klondike in cross 85, and a 1:2:1 segregation was observed in the  $F_2$ .

Rind toughness (*T*) of Golden Honey and Thurmond Grey was dominant to tenderness (*t*) of California Klondike, as shown in crosses 85 and 86 respectively. Segregation in the  $F_2$  was clearly 3:1, indicating a single-factor difference.

The crosses studied give no evidence of linkage of the following: (1) flesh color with seed-coat color; (2) flesh color with fruit-skin color; (3) flesh color with rind toughness; (4) fruit-skin color with rind toughness; (5) seed-coat color with rind toughness; (6) skin color with seed-coat color.

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## RELATIVE GROWTH AND HEREDITARY SIZE LIMITATION IN THE DOMESTIC FOWL<sup>1</sup>

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THE PROBLEM OF SIZE INHERITANCE has been extensively studied by many investigators since Mendel's demonstration of a genetic basis for stature in peas. Many different types of study have been undertaken by different investigators, working with diverse material. Probably, however, no single method of investigation is capable of yielding a complete solution to a problem as complex as that of size inheritance, and any individual approach can only be considered as giving additional evidence on the subject. In order to evaluate the contribution of such single approach, the problem as a whole must be visualized. Hence, when an attempt is made to present new evidence from a little-explored approach, an inventory of the work that has been done in this general field may be timely.

### REVIEW OF LITERATURE

In general, data obtained on various plants and animals show that the basis of size inheritance may in some cases be vested in single-factor differences and in other cases in a rather complicated scheme of multiple factors.

*Cases of Single-Factor Control of Size Differences.*—Cases of the first type are comparatively rare, although they extend throughout both the plant and the animal kingdom. In some instances it may be suspected that the single factors exert their influence at some critical stage of growth, affecting the development of certain organs which control size. Such, for instance, is the "dwarf" gene in mice, described by Snell (1929),<sup>3</sup> which was shown by Smith and MacDowell (1930, 1931) to affect the produc-

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<sup>3</sup> See "Literature Cited" at the end of this paper for complete data on citations, which are referred to in the text by author and date of publication.

tion of the anterior pituitary growth hormone. Kemp (1933), who described the histology of the normal and "dwarf" hypophyses, found differences in their development. The dwarf condition in rats, described by Lambert and Sciuchetti (1935), which was also found to be monofactorial in nature, may well be due to similar differences. Although hypophyseal implants in this case did not alleviate the dwarf condition, these workers suggest an endocrine basis for the reduction in size. The case of the dwarf fowl (Mayhew and Upp, 1932; Upp, 1934) has not found an explanation as yet though its inheritance on an autosomal monofactorial basis is indicated. Sollas (1909, 1914) reported an achondroplasialike dwarf character in the guinea pig, which was recessive in its inheritance, while Greene, Hu and Brown (1934) reported a lethal dwarf mutation in the rabbit.

In *Drosophila* single genes affecting size have been described, notably the "giant" of Bridges and Gabritschevsky (1928), while several cases have been recorded in plants (for example, Miyazawa, 1921; Stanton, 1923; Elders, 1928; Clarke, 1931; Sieglinger, 1933; Ramiah, 1933; Harland, 1934).

*Cases of Multiple-Factor Control of Size Differences.*—The second type of size inheritance, however, is one of particular interest. Sinnott and Dunn (1935) gave an excellent review of the literature and of the status of the problem to date. They list five lines of evidence for the multiple-factor control of quantitative characters in general and of size in particular. Before presenting these here, it may be noted that at least one worker (Castle, 1929, 1933b) has questioned the concept of chromosomal control of size factors. On the basis of the results of Moore (1933), who found maternal rates of cleavage in hybrids of lower marine forms, even when the zygotes were denucleated, Castle suggests the existence of a cytoplasmic control of size inheritance. The rejoinder of Dobzhansky (1934), who shows that the multiple-factor hypothesis fits best to most of the data on hand and the evidence obtained by Green (1930 *et seq.*), Feldman (1935), and Maw (1935), to be referred to later, disposes fairly well of Castle's objections to the theory of genic control of size. Yet as Marshak (1936) points out, growth rates may be the result of interaction of genes with cytoplasm; and because of the possibility of argument in this field, particular emphasis is laid on the evidence for the theory as outlined by Sinnott and Dunn. The five major lines of evidence pointing to the validity of the multifactorial concept are listed as follows:

1. The variability of the  $F_2$  generation is greater than that of the  $F_1$ .
2. Genetical distinctness of the  $F_3$  progenies from various points on the  $F_2$  curve of distribution.

3. Linkage of some genes for quantitative characters with those usually referred to as qualitative characters.

4. Evidence from *Datura*, that each chromosome may have a bearing on some quantitative trait.

5. Occurrence of the phenomenon of heterosis.

Most of the above conditions have been observed in many different forms, although only in a very few cases have definite factorial bases for size differences been assigned. The work of Lock (1906) and Emerson and East (1913) on maize, of Keeble and Pellew (1910) and de Haan (1927) on peas, of Nilsson-Ehle (1909), Freeman (1919), and Tingey (1932) on wheat, of Emerson (1910), Sax (1924) and Frets and Wan-rooy (1934, 1935) on beans, and many others can be cited from the plant kingdom.

In some cases definite single factors have been discovered. Thus Frost (1923) found linkage of size genes with color genes in the radish. Lindstrom (1928) succeeded in isolating a major gene affecting fruit size in tomatoes, while Currence (1932) also located a size factor on a marked chromosome in the same species. Smith (1935) reported linkage of one of the factors affecting corolla size in tobacco with a color factor, and Weetman (1935) found association of rind marking with size of watermelons. A question of importance was raised in some of this work as to an additive or a multiplicative effect of size genes, with Crane and Lawrence (1933) and Lindstrom (1935) presenting evidence for the first type of action, and Dale (1928) and Kaiser (1935) for the second.

*Size-Inheritance Studies with Animals.*—Very numerous too are the investigations with animals, most of which, however, were purely empirical types of study. Linear or mass measurements were classified phenotypically and in most cases blending inheritance was found to exist. In the larger animals, Spöttel (1932) described size inheritance in crosses of cattle with related forms, and Hammond (1920) worked on breed crosses in cattle. In swine Wellman (1913), Hammond (1922), Schmidt, Lauprecht, and Winzenburger (1934) presented evidence of the same general type. Hammond (1921), Ritzmann (1923), Davy (1927) and Spöttel (1932) worked with sheep. In laboratory mammals, Castle (1909 *et seq.*), MacDowell (1914), Punnett and Bailey (1918), Kopeć (1924), Pease (1928) and Robb (1929a) presented results of crosses between various breeds of rabbits; Castle (1916), also worked with guinea pigs as did Detlefsen (1914), while Livesay (1930) crossed different strains of the Norway rat. Vetusani (1930), Green (1930 *et seq.*) and Feldman (1935) gave evidence on size inheritance in mice. Here Green (1931a) was able to demonstrate linkage of one of the size factors

with a gene for a coat-color character, while Feldman also isolated a major gene for size. Castle, Gates, and Reed (1936) propose that the genes for some of the coat characters themselves have an effect on size. In man, Davenport (1917, 1924) made the most exhaustive study on the subject of inheritance of stature.

Domestic fowl were a popular material in this field with Ghigi (1909), Punnett and Bailey (1914), May (1925), Sokolov (1926), Ghigi and Taibell (1927), Kopeć (1927), May and Waters (1927), Kondyrev (1928), Danforth (1929), Warren (1930), Jull and Quinn (1931), Waters (1931, 1934), Lerner and Asmundson (1932), Axelsson (1933), Zorn and Krallinger (1934) and Lauth (1935), describing a variety of crosses from the standpoint of size inheritance. The same problem was studied in ducks by Phillips (1912, 1914) and by Goldschmidt (1913). A most important contribution was that of Maw (1935) who found one of the factors responsible for size differences between Sebright Bantams and Light Brahmans to be sex-linked. This work will be discussed in greater detail in a later section.

While variable results were obtained by the different workers, it became evident from the mass of data accumulated that further progress by these means in all probability would not lead to the complete solution of the problem. Several other approaches were tried and some of them yielded valuable leads.

*Studies on the Morphological Basis for Size Differences.*—Such, for instance, was the work designed to determine the morphological basis for size differences. Obviously individuals differing in total size must differ either in number or size of cells. Of the reports dealing with the subject in addition to the literature reviewed by Sinnott and Dunn (1935)—which includes among others the work on rabbits of Painter (1928), Castle and Gregory (1929) and Gregory and Castle (1931)—the work of Keller (1933) on the fowl may be cited as showing that cell-number and not cell-size differences are responsible for differences in total size.

On the other hand, there is also work indicating that cell size may in some cases play a part. Rensch (1923) has given a thorough review of this subject, as have Sinnott and Dunn, citing a number of workers who found that in polyploid series body size was roughly proportional to cell size. Rensch, in contradiction to the later work of Keller (1933), found not only cell-number but also cell-size differences between large and small races of fowl, while Dobzhansky (1929) found that reduced wing size in "miniature" *Drosophila* was due to smaller cells. Houghtaling (1935) concluded that fruit size in the tomato depends on both cell size and cell number, while Sinnott (1935b) states that in some cases, such

as that of the watermelon, size differences are due to a great expansion of a relatively small number of cells, but in others, such as the mammoth pumpkin, to a lesser expansion of a greater number of cells.

From this and other evidence many of the workers have concluded that ultimate size of an organism is determined early in its development. Thus, Ford and Huxley (1927) state that defined adult absolute size was inherited and hence an interpretation follows that developmental processes are a function of this heritable character. In keeping with this, Castle and Gregory (1929) and Gregory and Castle (1931) observed differences in the rate of cell proliferation in rabbit embryos of races with different adult sizes. Blunn and Gregory (1935) confirmed this for chickens.

As far as some of the chemical factors involved are concerned, their expression may also be a function of the final weight. Thus Gregory and Goss (1933a *et seq.*) found differences in the glutathione concentration of small and large races of rabbits at the time of birth. Similar findings in the embryos of chickens are reported by Gregory, Goss, and Asmundson (1935).

Lerner and Asmundson (1932) and Asmundson and Lerner, (1933, 1934) attempted to find differences in the rate of growth leading to the difference in final size in fowls and established early differential rates of growth in various breeds. Of the other studies on the bases of size difference, the work of Kaufman (1927 *et seq.*) on pigeons and chickens, showing that the final size difference between these two species is due to an original size difference, and that of Byerly (1930) on chicken embryos must be mentioned, as well as the interpretation of the latter's work by Castle and Gregory (1931), who demonstrated that crossbred embryos grow faster than purebred ones.

Many of the papers cited are important contributions to the problem, yet the status of the question is far from satisfactory at this time. While the multiple-factor theory can probably satisfy the results obtained from most of the crosses, especially if allowance is made for factor interaction or if Rasmusson's (1933) indication that 100–200 genes should be considered as involved in the segregation of quantitative characters is accepted, the mode of action of these factors on the dynamics of size differentiation is not at all understood.

*General and Special Size Factors.*—A rather important question is whether or not size genes are general in their effect. A controversy on this subject took place between Castle (1914 *et seq.*) and Sumner (1923 1924), with the former taking the stand that factors for size are mostly general in their effect, while the latter assumed that there are factors

affecting only the size of parts. The work of Davenport (1917, 1924) and of Mjoen (1923, 1926), reporting disharmonies in proportion found in human interracial crosses, was cited as supporting the latter point of view. The data of Eckles and Swett (1918) may be by inference interpreted to mean that factors affecting skeletal growth in cattle are common to all bones. Gregory (1933), also working with cattle, found that factors affecting linear development may be different from those affecting muscular development. Rumiantzev (1932), studying bone variability in rabbits, found a preponderance of general factors. Kopeć and Latyszewski (1930) give evidence for the existence of general factors in mice, while Green and Fekete (1933) and Green (1934) found both general and group factors in mouse crosses. Schkljar (1935) states that the growth of some organs in the chick embryo is "subject to general conformity to law."

The most illuminating of all is the work of Wright (1932) based on the extension of his original path coefficient analysis of general, group, and special factors (Wright, 1918). Using the data of Castle, he found that genetic differences in the size of rabbits were largely due to general factors and to a small extent to group and special factors. His analysis of the data of Dunn (1928) on the bones of inbred White Leghorn fowls revealed that here too general factors were predominant, but group and special factors were also of some importance.

In this problem special cases have to be distinguished from the general ones. Of frequent occurrence are the cases where genes affect only certain parts and organs, and these must be differentiated from the conditions prevalent in ordinary circumstances. Sinnott and Dunn (1935) list a number of these cases: brachydactyly; Ancon sheep; Creeper fowl; tail length in fowl, mouse, cat; ear length in mouse, sheep; hair length in rabbit, guinea pig, cat; comb in fowl; wing size, eye size, leg length, bristle length in *Drosophila*; and many cases in plants.

To this list may be added the genes for size of parts in the dog: short legs (Plattner, 1911; Wellman, 1916; Stockard, 1932), short tail (Klodnitzky and Spett, 1925), short head (Wriedt, 1929). Other cases include rumplessness in the fowl (Dunn, 1925; Landauer, 1928); probably an achondroplasialike condition in cattle (Crew, 1923; Mohr, 1929); the amputated condition in cattle (Wriedt and Mohr, 1928); hypotrichosis in mice (Loeffler, 1934) and other animals; atrichosis in mice (Brooke, 1926; Lebedinsky and Dauvart, 1927; Snell, 1931) and rabbits (Kislovsky, 1928); and ectromelus (Rabaud, 1914; Cuénot, 1928) in mice. Another interesting case is that of factors for thyroid size in ring doves, described by Riddle (1929).

In many of these cases it is obvious that while the manifestation of the gene involved is in only one organ or part, the effect is a general one. For instance, some of the genes when homozygous are found to be lethal. Such are the cases of brachyury and anury in mice (Dobrovolskaia-Zavadskaja and Kobozieff, 1930; Chesley, 1932, 1935; Ephrussi, 1933; Kobozieff, 1934), and the Creeper condition in the fowl (Landauer and Dunn, 1930). Reduced viability in short-tailed cats is reported by Schwangart and Grau (1931), and in short-tailed dogs by Klodnitzky and Spett (1925). Hunt, Mixter, and Permar (1933) showed that other effects are present in mice bearing the flexed-tail gene, and Kamenoff (1935) reported this condition to be the result of a general effect, associated with growth retardation and anemia.

The reason why such genes find their expression in certain parts of the body may be that their action takes place at what Stockard (1921) terms "critical moments" of growth for the parts concerned. Thus Kamenoff (1935) invokes this theory in explanation of the failure of cartilages to differentiate normally, resulting in the flexed tail with associated general effects in the mouse. This is also borne out by Danforth's (1932) production of rumpless fowls by fluctuations in incubator temperature at the probable time of most active differentiation of the posterior part. Of great importance is the contribution of Landauer (1934), who found that changes in the bones of Creeper fowls are secondary to a general growth retardation. This was confirmed by Fell and Landauer (1935) from studies of tissues *in vitro*.

The problem arises as to whether or not in normal individuals the differences in size of parts contributing to differences in total size are due to different genes or to the differential effect of the same genes on the rate of growth of these parts. The most advanced statistical method of studying the question of special factors, evolved by Wright (1932), does not answer this point. Robb (1935), from his study of equine skulls, claims that single-gene differences in size may produce a differential effect on the parts. In rats of the same size and presumably of a similar genetic constitution for total size, the rate of growth of some parts was found by Moment (1933) to be dependent on the body size, irrespective of age. It should be noted that these rats were kept under conditions promoting rapid or slow rate of total body growth at will. From this a suggestion may be made that general factors affect the growth of each part involved in this case in accordance with its respective growth capacity.

Investigation of the growth relation of parts in individuals differing in the pattern of growth of various organs may throw additional light on the problem. The application of the concept of heterogony, which is to

be discussed in fuller detail below, has not been utilized to any great extent in studies of hereditary size limitation. The work that has been conducted from this angle will be referred to in the discussion of results presented.

The present investigation was started with the object of analyzing the genetic control of the growth of parts in relation to the growth of the whole body. Of the three phases of growth, chemo-, histo-, and auxano-differentiation (Huxley, 1932) the last-named was considered to be most suitable for the application of available statistical methods because during it only quantitative growth changes in size and proportions may take place.

#### CHOICE OF MATERIAL

*Advantages of the Domestic Fowl.*—In studies of inheritance of quantitative characters in larger animals, the domestic fowl (*Gallus gallus domesticus*) forms particularly favorable material. This is largely because control of certain environmental conditions is possible in this species. Thus a large number of siblings may be hatched on the same day and raised uniformly, without being subject to such limitations as small families or to environmental differences due to differences that occur in mammals, such as the number in a litter and the maternal state of nutrition. More comparable figures on various aspects of growth can be obtained by standardizing the feeding and other environmental factors.

From the standpoint of studying parts, the domestic fowl is probably on a par with the laboratory mammals, since fairly extensive data are available on the post-hatching growth and relative size of organs and structures. The work of Latimer (1924, *et seq.*) particularly, as well as the data of Zaitschek (1908), Latimer and Pedersen (1923), Souba (1923), Mitchell, Card, and Hamilton (1926, 1931), Koch and Dyman (1934), and Hopkins and Biely (1935) may be cited as giving pertinent data on the subject.

*Choice of Breeds.*—In attempting the investigation of the genetic basis of the growth of parts in relation to the whole, two possible avenues of approach present themselves. The hybrids investigated may be (1) between breeds or strains of different size, or (2) between breeds of the same size but of varying proportions of parts. The first approach would introduce a variable in the form of total size and consequently may complicate the methods of investigation. The second method theoretically should provide a sounder basis for comparison of parts and for establishing any possible genetic differences in their relative growth, since the variable in the form of total body size would be in this case held constant.

It was believed at the outset of this investigation that the Plymouth Rock and the Minorca breeds of the domestic fowl would answer specifications for material for the latter method of approach. In appearance the two breeds differ markedly, the first one being a fairly low-set, rounded-out bird, while the second is high-set and somewhat angular in body shape. The standard weights of these breeds, as determined by the American Standard of Perfection (American Poultry Association, 1930) are as follows:

	<i>Plymouth Rock</i> <i>lbs.</i>	<i>Minorca</i> <i>lbs.</i>
Cockerel . . . . .	8	7½
Cock . . . . .	9½	9
Pullet . . . . .	6	6½
Hen . . . . .	7½	7½

These weights represent the ideal for exhibition and the objective of attainment by breeders of the two respective breeds of fowl. Although considerable variation exists within the breeds, it may be said that most of the well-established strains have characteristic weights, deviating only within reasonable limits from the standard, this deviation being usually in the direction of lower weights.

As far as the breeding behind the two varieties is concerned, evidence on hand indicates that they have no common ancestors since records have been kept (Brown, 1906; Robinson, 1924). The Minoreas were introduced from the Mediterranean into England about 1780 and appeared in the United States as an importation from that country in 1884 or 1885. The Plymouth Rock by that time had been exhibited for more than ten years, originating in America from crosses of English and Asiatic fowls.

While the total size of the two breeds is approximately the same, the assumption that differences exist in such factors as bone length and muscular development had, to judge from the external appearance of the birds, some basis. The selection of the particular structures to be studied was governed by various considerations, such as the possible significance of differences and the ease of dissection. The original outline of the experiment provided for preliminary studies on several organs and parts. After some work with the material, however, it was found impracticable to study most of the originally proposed structures, and the final choice fell on the pectoralis major and the long bones of the leg. The actual material used and the dissection technique employed is described in the next section.

## MATERIAL AND METHODS

*Stock Used.*—The original parental stocks used for this investigation came from three sources. Twelve yearling hens and 2 cockerels of known pedigree were purchased from a California breeding farm to form the foundation Barred Plymouth Rock stock. Four hens of the University of California flock and 6 hens and 2 cockerels obtained from a southern California breeder formed the Black Minorca foundation stock. These breeds were first mated in two pens in 1934, so as to give purebred and reciprocal cross progeny. These birds and some of their progeny were remated the following winter in six pens to produce additional purebreds and first crosses, as well as backcrosses to both of the parental breeds to the total number of ten matings. The pens were arranged in pairs and the males interchanged between the paired pens, so as to obtain samples representative of the population and not of individual families. All of the chicks were individually pedigreed.

Altogether nine hatches of chicks were used, four in the first and five in the second breeding season. These were raised under practically identical conditions at the University poultry plant. The only deviation from the standard brooding system was practiced in the first three hatches of the first year, which were kept in a battery brooder for the first five weeks of their life. The standard University of California mash was used and did not vary throughout the whole of the investigation. It consisted of the following ingredients:

	<i>Per cent</i>		<i>Per cent</i>
Ground yellow corn.....	52.5	Dehydrated alfalfa.....	5.0
Ground wheat.....	10.0	Ground oystershell.....	1.5
Wheat bran.....	15.0	Bonemeal .....	0.5
Fish meal.....	9.5	Salt .....	0.5
Dried milk.....	5.0	Cod-liver oil .....	0.5

Whole grain in the proportion of 2 parts corn to 1 part wheat was fed beginning at four weeks of age and gradually increasing in amount until, at sixteen weeks of age, half of the feed consumed consisted of the whole-grain mixture.

The birds were weighed at regular intervals, and observations were made on the color of plumage and the rate of tail-feather growth. These, however, were found to be of little value in analyzing the results, both being controlled by genes located on the sex chromosome, while no sex-linkage was found in the other data studied.

At various ages, groups of birds were killed and dissected in accordance with the technique described below. All of the birds surviving to the ages at which random selections for killings were made, with the

exception of sick or otherwise abnormal individuals, were eventually used for that purpose. In all, seventeen dissection series were carried through.

Table 1 records the number of chicks used, arranged according to the age of the birds at the time the dissections were made. It also shows the

TABLE 1  
DISTRIBUTION OF BIRDS DISSECTED BY MATING, SEX, AND AGE

Mating*	Sex	Group No.	Age in Weeks									Total	
			2	4	8	9	10	12	14	16	20		
Barred Plymouth Rock.....	{♂	1	1	8	3	0	0	3	0	20	8	3	46
	{♀	2	1	11	2	0	1	3	1	10	3	10	42
Black Minorca	{♂	3	0	12	2	1	2	2	2	8	2	6	37
	{♀	4	1	9	2	2	0	4	0	8	1	14	41
1×4.....	{♂	5	3	12	5	2	0	1	0	2	3	10	38
	{♀	6	1	12	2	4	1	3	1	3	2	4	33
3×2.....	{♂	7	7	22	1	6	8	5	4	13	1	19	86
	{♀	8	4	17	2	4	3	3	4	7	4	9	57
1×6.....	{♂	9	4	11	1	2	5	6	1	4	2	0	36
	{♀	10	5	8	4	5	1	6	3	1	3	0	36
1×8.....	{♂	11	0	5	0	2	3	2	3	3	2	0	20
	{♀	12	2	4	1	0	2	3	2	1	2	0	17
3×6.....	{♂	13	3	12	3	2	6	3	5	2	0	0	36
	{♀	14	2	10	4	5	3	8	2	3	3	0	40
3×8.....	{♂	15	2	10	0	3	2	4	2	2	1	0	26
	{♀	16	3	9	2	3	2	2	3	4	0	0	28
5×2.....	{♂	17	2	7	2	4	3	5	5	5	2	0	35
	{♀	18	0	11	3	2	4	4	3	4	5	0	36
7×2.....	{♂	19	2	11	4	4	1	7	4	3	3	0	39
	{♀	20	4	5	7	3	1	4	1	3	3	0	31
Total.....	..	..	47	206	50	54	48	78	46	106	50	75	760

\* The figures refer to the numbers of the groups used as parents for the various matings.

distribution by sexes of the ten types of crosses. As indicated in the table, the smallest number represented per cross is 17 for group 12, while the largest is 86 for group 7.

**Dissection Technique.**—The dissection technique was practically identical in all of the seventeen series, with the exception of the first two, when measurements and weights in addition to those reported were taken. The details of the procedure were as follows: the birds to be killed were starved from the afternoon of the day previous to the day of killing

(birds in series 13 were fed grain early on the morning of the killing through an oversight of the attendant). On the morning of the day of dissection the birds were weighed individually and then killed by breaking the neck. The head was severed from the body and the carcasses were suspended by the legs for a period of a quarter of an hour or over to allow the drainage of blood. After this the birds were dissected in random order. The skin with the feathers was removed from the right side of the body. The right leg was removed at the acetabulum, properly marked for identification, and preserved for further treatment.

A sharp scalpel was used to remove the right tensor patagii muscles. The right pectoralis major was then dissected out with a scalpel. An incision across the insertion on the proximal end of the humerus was followed along the clavicle and down the keel of the sternum to the tip of the xiphoid process. This freed the origin of the muscle and permitted following the outside edge of it with a scalpel along the line marked by a layer of fat separating the pectoralis major from the obliquus abdominis externus. An assistant with a container of a known tare weight then obtained the gross weight of the muscle and container. In the last three series of dissections no container was used, but the various organs were weighed on a small piece of cellophane of known weight.

While the assistant was engaged in weighing the pectoralis major, the viscera of the bird were exposed and the liver dissected out. The gall bladder was cut off and the liver dried with an absorbent towel to remove the excess of blood. The liver was then weighed in the same manner as the pectoralis major, although data thus obtained are not being reported here.

The legs that were kept were roughly cleaned and then treated by a technique similar to the one used by Schneider and Dunn (1924), which involved boiling them in an alkaline solution and further cleaning. In a number of instances the bones could not be measured, usually because of overboiling the bones, but occasionally a bone was broken in the process of cleaning.

*Measurement and Weighing.*—Live weights of chicks up to four weeks of age were recorded to the nearest gram. Older birds were weighed to 10 grams. In the first and second series of dissections the weights of parts were recorded to 1 mg. In subsequent series the weighings were either made with an accuracy of 0.01 gram or 0.1 gram, according to the size of the parts studied. For the measurement of bones, vernier calipers calibrated to 0.01 mm were employed in the case of birds whose longest bone (tibiotarsus) did not exceed 75 mm. For longer bones a simplified method was used. The bones were placed on a millimetric ruler, in iden-

tical position for each bird and the distance between two farthest points on each bone recorded to 1 mm.

Every dissection and every bone measurement was made by the writer in order to equalize the personal factor. This factor entered to a slight degree in the recording of weights, since five different assistants were engaged for that purpose during the investigation. However, since the sensitivity of the balances employed extended to a higher degree of precision than that used in recording the weights, the personal factor may be considered as having been held at a minimum.

The data thus obtained were then subjected to a statistical analysis.

### RELATIVE GROWTH

*Growth of a Part in Relation to the Whole.*—The measurement of parts with respect to the whole forms a field of embryology and anatomy, which found new significance with the demonstration of the relation of the part to the whole by Huxley (1924, 1932). As Needham (1934) points out, both Dubois (1897) and Lapieque (1898) have approached the formulation proposed by Huxley, but it was the latter who gave an impetus to the collection of new data and to the re-examination of old. Considerable information is available on the subject in many forms of plant and animal life, and very able summaries of data gathered are given by Needham (1931) and Huxley (1932). The work on the domestic fowl, however, is rather limited, so far as the application of the simple formula of Huxley expressing the relation of the part to the whole is concerned. Most of that work has been done on embryological material, the data having been summarized by Needham (1934). The anatomical work on the posthatching growth of parts of the fowl was mentioned in an earlier section, but none of the workers concerned approached the subject either from the standpoint of possible breed differences or from the standpoint of what is termed heterogonic relation.

Obviously the growth of the organism as a whole is the sum total of the growth of its parts, while form or shape of the organism is the result of differential rate of growth of the parts, or differences in length of the growing period.

The genetics of shape has been studied particularly in plants, and the literature on the subject has been reviewed by Sinnott and Dunn (1935) and by Kaiser (1935). Most interesting results were obtained by Sinnott (1935a) and his associates (reviewed by Kaiser and by Sinnott and Dunn), indicating inheritance of shape of fruits of some plants irrespective of weight. Thus in *Cucurbita pepo*, Sinnott (1931) found that the particular dimensions of the fruit are a result of interaction of size and

shape factors, which are independent of each other. Sinnott and Dunn also point out that the genetic control of shape may be vested in (1) relative rates of cell division in different regions of body or organ, (2) cell shape, (3) plane of cell division.

It is probably the particular application of the first point that is involved in this investigation. The genes being dealt with are rate genes, which are factors controlling development and determining growth gradients, similar to Goldschmidt's rate genes in the sex determination of *Lymantria* (1927). Pertinent instances are that of the inherited differential growth rates between polar and equatorial dimensions in the fruit of the tomato (Houghtaling, 1935) and that of single major genes controlling relative dimensional growth rates in the fruit of peppers (Kaiser, 1935).

*Mathematical Expression of Heterogony.*—The rate of growth of a part relative to the whole has been termed "heterogony" by Pézard (1918) and a general mathematical expression formulated for it by Huxley:

$$y = bx^k, \quad 1$$

where  $y$  represents the part and  $x$  the whole, while  $b$  and  $k$  are constants. Sometimes the equation is written as

$$y = b(x-y)^k \quad 2$$

so as to relate the part to the remainder of the whole. This is done to avoid the absurdity of the part's eventually exceeding the whole when it grows at a faster rate. However, since growth is not infinite and the difference in  $k$  between the two cases is found to be very small, equation 1 has been accepted by most workers. Needham (1934) has pointed out that the equation cannot have a true physical meaning because it would not satisfy the theory of dimensions. This follows from the fact that if  $x$  is a mass,  $x^k$  cannot be unless  $k=1$ , and the requirement of the theory of dimensions that the dimensions on the two sides of the relation should be equivalent is not satisfied. However, this does not invalidate the usage of the heterogonic formula for purposes of comparison.

When  $k$  is higher than unity in value, the part is growing at a more rapid rate than the whole and the condition is termed positive heterogony. When  $k$  is less than unity, negative heterogony is indicated. Dawes and Huxley (1934) suggest the terms "hypergony" and "hypogony" to describe the respective cases, while "isogony" has been frequently used for the cases where  $k=1$ .

Robb (1929b) has drawn an analogy between the heterogonic growth relation and the conditions found when a solute is placed in two immisci-

ble solvents in contact with each other. The distribution of the solute to the solvents will occur in a certain ratio, known as the partition coefficient. Robb suggests that the distribution of growth essentials between tissues is analogously controlled by  $k$ , the partition coefficient of growth.

Equation 1 represents a logarithmic straight line in which  $k$  is the tangent of the angle between the straight line and the positive axis of abscissas and  $\log b$  is the intercept on the axis of ordinates.

Several methods of determining the values of the parameters of this straight line are available. A crude determination may be readily made by graphic means, by using a tangent meter. A more exact solution is possible by applying the method of least squares. As Feldstein and Hersh (1935a) point out, there are two ways of doing so. The first way can be used when arithmetic values of the original data are used. If  $w$  is the frequency weight, two observation equations are obtained

$$y^2w (\log y - \log b - k \log x) = 0 \quad 3$$

and

$$\log x [y^2w (\log y - \log b - k \log x)] = 0 \quad 4$$

From these, Feldstein and Hersh obtain the solution for  $k$  in the following form :

$$k = \frac{\Sigma y^2w (\Sigma y^2w \log x \log y) - (\Sigma y^2w \log x) (\Sigma y^2w \log y)}{\Sigma y^2w [\Sigma y^2w (\log x)^2] - (\Sigma y^2w \log x)^2} \quad 5$$

The other way of arriving at the value of  $k$  is to apply the least-squares method to logarithms of the original data. By a simple logarithmic transformation of equation 1 :

$$\log y = \log b + k \log x, \quad 6$$

which represents the regression line of  $\log y$  on  $\log x$ ;  $k$  can be solved by the ordinary regression-coefficient equation :

$$k = r \frac{\sigma_{\log y}}{\sigma_{\log x}} = \frac{n \sum \log x \log y - \sum \log x \sum \log y}{n \sum (\log x)^2 - (\sum \log x)^2}, \quad 7$$

where  $n$  equals the total number of individuals for which the determination is made.<sup>4</sup>

Feldstein and Hersh (1935a) applied both equations 5 and 7 to Hersh's (1931) data on facet number in different bar-eyed stocks of *Drosophila*, and although the values obtained by the two methods were

<sup>4</sup> Since this paper went to press Oscar W. Richards of Yale University has kindly called my attention to a paper by Schmalhausen (1931), which, antedating Feldstein and Hersh, gives a least-squares solution for  $k$  as well as formulas for the standard errors of  $k$  and of  $\log b$ .

different, the trends were found to be similar. They were not able to establish criteria for deciding which is the preferable method. For comparative purposes it may be imagined that the method which proves to be less laborious could be chosen for the data herewith presented; and the logarithmic method, embodied in equation 7 was found to be such.

The value of the intercept of the regression line can be obtained by substitution into one of the normal equations and solution for  $\log b$ . Another parameter which can be used is one designated by  $\log a$ . From equation 6:

$$\log b = \bar{\log y} - k \bar{\log x}. \quad 8$$

Substituting into equation 6:

$$\log y = \bar{\log y} - k \bar{\log x} + k \log x, \quad 9$$

or

$$\log y = \bar{\log y} + k(\log x - \bar{\log x}); \quad 10$$

$k$  remains here the same as in the original equation 1, while the new parameter  $\log a$  is the mean value of  $\log y$ :

$$\log a = \bar{\log y} = \frac{\sum \log y}{n} \quad 11$$

The standard error of  $k$  is in accordance with Feldstein and Hersh (1935b):

$$SE_k = \frac{\sigma_{\log y} \sqrt{1 - r^2_{\log x \log y}}}{\sigma_{\log x} \sqrt{n-2}} = \sqrt{\frac{n \sum \log^2 y - (\sum \log y)^2}{n \sum \log x^2 - (\sum \log x)^2} - k^2}, \quad 12$$

making allowance for the number of degrees of freedom. The reliability of the value of  $k$ , or the scatter of the observation data around the straight line defined by  $k$  and by  $\log a$ , can be determined from the usual statistical relation between a constant and its standard error. Equations 7 and 11 were used for the determination of the constants and equation 12 for the standard error of  $k$ . The standard errors of  $\log a$  are not presented.

Hersh (1931) has presented evidence that there is a definite relation between the values of  $b$  and  $k$  in the bar-eyed stocks of *Drosophila*. He found that the changes in  $b$  and  $k$  follow the exponential function:

$$b = Be^{-rk} \quad 13$$

where  $r$  is the relative rate of decrease in  $b$  per unit change in  $k$ , and  $B$  is the value of  $b$ , when  $k$  approaches zero. The uniform covariability of

$b$  and  $k$  may have a possible biological meaning, although Lumer (1936) questions equation 13 on mathematical grounds. Neither Huxley (1932) nor Needham (1934) attached any biological significance to the value of  $b$ . This may be an unnecessarily extreme point of view, since  $b$  in reality is the value of  $y$  when  $x$  equals unity and may have a distinct bearing on the preceding developmental history. However, since no ready interpretation of  $b$  is available,  $\log a$  is presented in this paper as being directly available from the observation data. The value of  $\log a$ , of course, can be considered only as a parameter defining the regression equation, since it will be affected by the chance distribution of the various groups in different age classes at the time the observations were taken. Hence its only use for the present purposes is as a parameter, which with  $k$  can be used to construct the regression equation, although it also characterizes each group.<sup>5</sup>

When the relation of skeletal growth to total body weight is considered, linear measurement is used for one coördinate and mass measurement for the other. However, weight and length of bones are related:

$$\text{Weight} = C \text{length}^3, \quad 14$$

or written in the form of:

$$y = Cy^3 \quad 15$$

The calculation of the coefficients of heterogonic growth has been made on the basis of an equation of the type

$$y = bx^k \quad 1$$

However, since only linear measurements were available on bones first

$$y_1 = b_1 x^{k_1} \quad 16$$

was calculated, where  $y_1$ , as in 15 represents bone length. Substituting 15 in 16, and cubing:

$$y = C b_1^3 x^{3k_1} \quad 17$$

Here  $\log C + 3 \log b_1$  is the intercept on the axis of ordinates when the equation is transformed into logarithmic form, while

$$3k_1 = k \quad 18$$

---

<sup>5</sup> Since this was written my attention has been drawn to a paper by Richards (1935), wherein a critique of the concept of  $k$  is presented. The objections cited include the variability of  $k$  during the growing period, the inadequacy of the graphic method of estimation of  $k$  values, and the oversimplification of growth processes coincident with its use. However, comparison of  $k$ 's when they are considered as pure dimensionless numbers arrived at under identical conditions is permissible.

Consequently to obtain the true coefficient of heterogonic growth of the bone lengths, the values of  $k_1$  should be multiplied by 3.

It has been mentioned that owing to faulty technique some of the bones were not available for measurement. Hence not all of the individuals dissected were used to determine the value of  $k_1$ , while other birds used in leg-bone computations lacked the pectoralis-major weight. Hence, a

TABLE 2

NUMBER OF INDIVIDUALS USED FOR THE DETERMINATION OF RELATIVE GROWTH CONSTANTS BY GROUPS

Mating	Males				Females			
	Group	Relation studied			Group	Relation studied		
		Pectoralis major and body weight	Leg bones and leg length or body weight	Pectoralis major weight and leg length		Pectoralis major and body weight	Leg bones and leg length or body weight	Pectoralis major weight and leg length
Barred Plymouth								
Rock.....	1	45	43	42	2	42	39	39
Black Minorca	3	35	29	29	4	41	37	37
1×4.....	5	38	35	35	6	33	28	28
3×2.....	7	86	82	82	8	57	51	51
1×6.....	9	35	36	35	10	35	34	33
1×8.....	11	20	20	20	12	17	17	17
3×6.....	13	36	35	35	14	40	40	40
3×8.....	15	26	26	26	16	28	28	28
5×2.....	17	35	35	35	18	36	34	34
7×2.....	19	39	39	39	20	29	31	29

discrepancy arose in numbers for the various determinations. Table 2 presents the actual number of birds involved for each group for each calculation.

### PECTORALIS MAJOR

Figure 1 presents the logarithmic plot of the weight of the pectoralis major against body weight for the male progeny of the different matings. It is used to illustrate plots made for all of the structures studied for the two sexes, where individual observations were used. A straight-line relation is shown in this figure, indicating that  $k$  is fairly constant for the post-hatching period of growth. Moreover, it can be seen that in all of the ten types of matings a uniformity exists in the logarithmic ratio of the pectoralis major to the body weight. The figure in itself, however, does not give a clear indication as to whether or not there are any definite differences in the value of  $k$  between crosses. Any minute but significant differences would not show up in graphic form.

Table 3 gives the actual values of  $k$ , together with their standard errors, for both the males and the females. It should be noted that in the calculation of the standard errors in some cases cumulative differences in decimal places brought the value under the square root in equation 12 to zero or even to a negative number. In these cases, the standard error

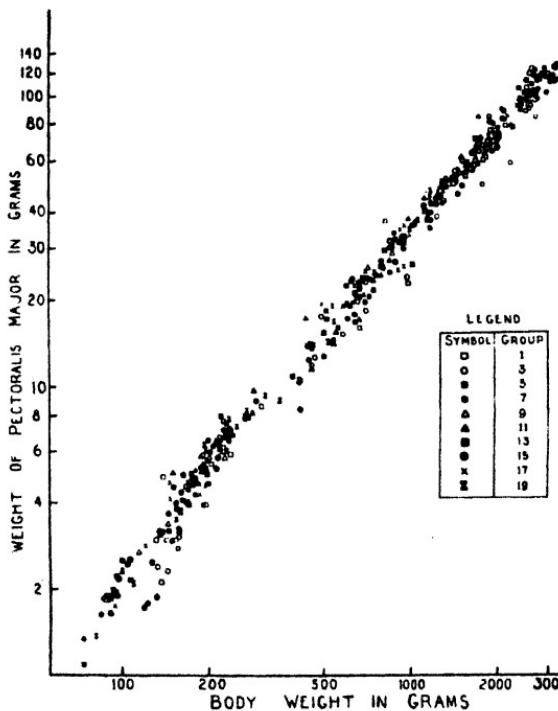


Fig. 1.—Weight of the pectoralis major of the males plotted against body weight on a double log grid. For key to group numbers see table 1.

was estimated on the basis of a minimum difference between the two expressions under the square root in equation 12. It may be readily observed that while the range of the values varies from 1.148 for the purebred Plymouth Rock males and females to 1.249 for the purebred Minorca males, the standard errors are of such a magnitude as to render no difference statistically significant. In these extreme cases the actual standard errors are available and not the estimated values as stated above.

So far as the differences between the sexes are concerned, in most cases the females showed a higher  $k$  value than the males of the corresponding mating, though at least one case of the reversed condition can be ob-

served. If it can be said that all of the  $k$  values for the same sex are essentially the same and the apparent differences are due to chance variation, no significance can be attached to the difference between the sexes since the actual values overlap considerably throughout the series of 20 groups. However, when the males and females in the various groups are paired and analyzed by Student's method, a definite tendency may be noted favoring a higher  $k$  value for the females. The value of  $z$  is 0.731, which is approximately equivalent to 34:1 odds on significance. This would in-

TABLE 3  
RELATIVE GROWTH CONSTANTS FOR THE WEIGHT OF THE PECTORALIS MAJOR WITH  
RESPECT TO BODY WEIGHT

Mating	Males			Females		
	Group	$k$	$\log a$	Group	$k$	$\log a$
Barred Plymouth Rock	1	1.148±0.130	1.596	2	1.148±0.125	1.419
Black Minorca.....	3	1.249±0.141	1.351	4	1.231±0.040	1.419
1×4.....	5	1.167±0.032	1.348	6	1.183±0.114	1.269
3×2.....	7	1.199±0.110	1.336	8	1.201±0.179	1.280
1×6.....	9	1.187±0.084	1.178	10	1.244±0.110	1.150
1×8.....	11	1.172±0.232	1.438	12	1.248±0.055	1.201
3×6.....	13	1.201±0.095	1.074	14	1.210±0.176	1.173
3×8.....	15	1.179±0.126	1.112	16	1.246±0.138	1.097
5×2.....	17	1.176±0.110	1.311	18	1.196±0.137	1.262
7×2.....	19	1.201±0.095	1.261	20	1.204±0.184	1.236

dicate that if the  $k$  values were calculated for the entire population and separately for the two sexes, the  $k$  for the females would be higher than that for the males.

Though no data exactly comparable can be found in the literature, it may be of interest to note that Mitchell, Card, and Hamilton (1926), working with White Plymouth Rocks, found that the percentage of flesh and fat in the carcass is higher in females than in males of the same weight. A similar condition was found by the same workers (1931) to prevail in White Leghorns. When the relative increase in the weight of flesh and fat in the carcass was calculated with respect to increase in body weight, they found that it was greater in cockerels than in pullets. The discrepancy here is probably due to the fact that at the time of hatch the standard of comparison of body weight was higher for the pullets than for the cockerels, while the reverse was true in later stages.

Irrespective of sex, the weight of the pectoralis major increased more rapidly than the weight of the whole of the body, and thus presented a case of hypergonia. Log  $a$  is also shown in table 3, and from this parameter and the value of  $k$ , the regression equation can be constructed for

each one of the twenty groups studied. With a similar range of weights for the parental crosses, it is highly significant that the equations do not materially differ from each other. In general, the Plymouth Rock is considered to be a better meat-type bird than the Minorca. Here, however, it may be seen that the amount of fleshing, so far as it can be judged from the weight of the pectoralis major, is the same in the two breeds. A comparison of the data in the two reports of Mitchell, Card, and Hamilton (1926, 1931) reveals that the Leghorn, a breed definitely not possessing

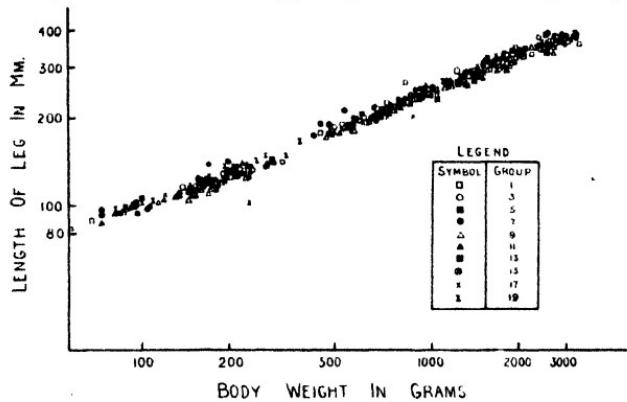


Fig. 2.—Leg length of the males plotted against body weight on a double log grid. For key to group numbers see table 1.

high meat quality, has at the same weights as the Plymouth Rock, a higher percentage of flesh. In this instance, however, the latter reaches a distinctly higher adult weight (Brody, 1926) and possesses a more rapid rate of growth (Asmundson and Lerner, 1934), which does not seem to be the case with the two breeds under comparison here. Thus from the practical point of view it may seem that the value of Minorcas as meat producers has been underestimated unless there is a difference in the growth of the two breeds with respect to time. Investigation of this point is impossible with the material available because of the relatively small numbers of individuals in each hatch.

#### LEG BONES

A logarithmic plot of the total leg length obtained by addition of separate arithmetic values for the femur, tibiotarsus, and tarsometatarsus against body weight is given in figure 2 for the males. The females show a comparable distribution, the plot not being included here, as in the case of the pectoralis major, for reasons of economy of space. It may be noted, however, that a slight flattening of the line at the extreme weight range

is observed in the females due to continued deposition of fat after linear growth has ceased. In general, the observations made about the pectoralis major may be repeated here. A straight-line relation is indicated and a uniform tendency in all of the ten groups can be noted.

Table 4 gives the values of  $k_1$ , for the whole leg as well as for the constituent bones in relation to body weights, while table 5 shows the values

TABLE 4

VALUES OF  $k_1$  FOR THE TOTAL LEG LENGTH AND LENGTH OF CONSTITUENT BONES IN RELATION TO BODY WEIGHT

Mating	Group	Total leg*	Femur*	Tibiotarsus*	Tarsometatarsus
Barred Plymouth Rock.....	1	0.394±0.076	0.365±0.077	0.405±0.063	0.411±0.077
	2	0.354±0.069	0.335±0.053	0.364±0.066	0.363±0.093
Black Minorca.....	3	0.399±0.028	0.361±0.044	0.420±0.142	0.410±0.118
	4	0.361±0.026	0.334±0.024	0.373±0.004	0.374±0.054
1×4.....	5	0.398±0.047	0.370±0.041	0.409±0.050	0.411±0.048
	6	0.384±0.053	0.363±0.049	0.393±0.050	0.391±0.069
3×2.....	7	0.399±0.032	0.373±0.032	0.408±0.032	0.419±0.032
	8	0.394±0.055	0.374±0.032	0.400±0.045	0.410±0.063
1×6.....	9	0.400±0.010	0.382±0.010	0.401±0.010	0.414±0.045
	10	0.396±0.032	0.384±0.045	0.396±0.010	0.411±0.045
1×8.....	11	0.410±0.077	0.372±0.077	0.418±0.084	0.433±0.095
	12	0.389±0.032	0.365±0.032	0.397±0.010	0.406±0.032
3×6.....	13	0.399±0.054	0.367±0.045	0.402±0.055	0.427±0.063
	14	0.411±0.055	0.375±0.285	0.417±0.055	0.430±0.077
3×8.....	15	0.411±0.045	0.384±0.182	0.414±0.063	0.433±0.105
	16	0.412±0.045	0.380±0.055	0.415±0.155	0.441±0.068
5×2.....	17	0.397±0.045	0.371±0.045	0.404±0.045	0.417±0.068
	18	0.417±0.045	0.381±0.045	0.430±0.032	0.439±0.032
7×2.....	19	0.410±0.063	0.380±0.055	0.413±0.063	0.433±0.071
	20	0.408±0.071	0.382±0.045	0.412±0.055	0.417±0.063

\* Standard errors below 0.032 are estimates.

of the other parameter of the regression equation. As already mentioned, the true coefficient of heterogonic growth can be obtained by multiplying  $k_1$  by 3.

The uniformity of the  $k_1$  values for the total length of the leg is very striking. Not only do the two parental breeds show the same logarithmic relation between leg length and total body weight, but apparently, as seen from the graphic representation, the actual leg length values are the same in the two breeds when individuals of similar weights are compared. The seeming difference in appearance of the legs of the two breeds

is evidently not due to differences in the auxano-differentiation of the bones nor to differential length. A possibility exists that it is either a postural difference or merely a superficial difference based on a different type of feathering in the region of the pelvic girdle. It is, of course, highly probable that other breeds of poultry show real differences in the

TABLE 5  
VALUES OF LOG  $a$  FOR TOTAL LEG LENGTH AND LENGTH OF CONSTITUENT BONES

Mating	Group	Total leg	Femur	Tibiotarsus	Tarsometatarsus
Barred Plymouth Rock . . .	{ 1	2.429	1.894	2.047	1.894
	{ 2	2.350	1.825	1.969	1.805
Black Minorca . . . . .	{ 3	2.392	1.858	2.007	1.862
	{ 4	2.377	1.849	1.994	1.839
1×4. . . . .	{ 5	2.341	1.812	1.957	1.801
	{ 6	2.295	1.775	1.908	1.752
3×2. . . . .	{ 7	2.345	1.817	1.958	1.808
	{ 8	2.316	1.794	1.934	1.775
1×6. . . . .	{ 9	2.250	1.730	1.862	1.711
	{ 10	2.234	1.718	1.848	1.688
1×8. . . . .	{ 11	2.339	1.816	1.952	1.802
	{ 12	2.249	1.729	1.866	1.704
3×6 . . . . .	{ 13	2.243	1.725	1.854	1.703
	{ 14	2.264	1.744	1.879	1.728
3×8. . . . .	{ 15	2.256	1.732	1.868	1.702
	{ 16	2.238	1.719	1.852	1.694
5×2. . . . .	{ 17	2.314	1.789	1.926	1.777
	{ 18	2.295	1.774	1.911	1.751
7×2. . . . .	{ 19	2.294	1.773	1.906	1.768
	{ 20	2.253	1.731	1.867	1.711

length of leg, but differences in the heterogonic relation between the leg and the body still remain to be determined in these cases.

Such a case is available in cattle, where Swett, Graves, and Miller (1928) compared the measurements of a Jersey cow with those of an Aberdeen Angus cow. The former exhibited longer leg bones than the latter but weighed less. Though the constant differential growth ratio cannot be determined from the data supplied by these workers, it is possible that in the Jersey the leg bones show a more positive heterogony than do those of the Aberdeen Angus. This, of course, is not definite evidence of a hereditary condition since the intercept of relation or the nutritional state of the animals may be the responsible factor.

Kopeć (1927) found that the Orpingtons have longer tibiotarsi and tarsometatarsi than Leghorns. The length of the bones of the hybrids was found to be in most cases closer to the longer-boned parent. However, the differences are small, the number of birds was small, and overlapping of ranges is indicated; furthermore, no tests of statistical significance are given.

Ghigi and Taibell (1927) have also reported that in an Indian Game  $\times$  Leghorn cross, the  $F_1$  tarsi were of the same length as that of the longer-boned parents. No actual figures were cited by them.

Lerner and Asmundson (1932) presented measurements of the tarsometatarsi of Ancona  $\times$  Light Sussex hybrids and backcrosses to parental breeds at twelve weeks of age. They found that there was a significant difference in the length of the bone studied between the two types of backcross progeny in favor of the Light Sussex  $\times F_1$  mating. However, these birds were also the heaviest and thus possibly the heterogonic relation of the two was the same. This is further supported by the fact that when the logarithmic ratios of the mean bone length to mean body weight as given in their paper are calculated, they are found to be practically identical for both types of backcrosses. The same is true for the data of Kopeć (1927) with respect to his parental breeds.

Thus the question as to whether or not conditions similar to those found by Swett, Graves, and Miller (1928) in cattle exist in the normal-sized breeds of domestic fowl remains open. The term "normal-sized" refers here and hereafter to breeds other than Bantams. The relation in Bantams was determined and will be discussed at length in a separate section.

It may be noted that when the tabular values of  $k_1$  are multiplied by 3 to obtain  $k$ , hypergonia is indicated for the whole leg and for its constituent bones. This holds true for all of the birds studied here irrespective of origin and confirms Latimer's findings (1927) that the rate of growth of the leg bones in the fowl is higher than the rate of body weight growth. A sex difference in the  $k_1$  value for leg length can also be observed here. Student's method of pairing shows that the odds for significance of this difference with a  $z$  value of 1.444 are over 1000 to 1. The sex difference here is in favor of the males, once more in accordance with the results of Latimer (1927), who shows that the male bones increase from hatching weight to maturity to a greater extent than do female bones, with the exception of the femur, where no difference was found.

The interrelation of the three bones may be best illustrated by the use of the coefficient of heterogonic growth with respect to total leg length rather than to body weight.

## GROWTH GRADIENTS IN THE LEG

The values of the exponent  $k$  for the femur, tibiotarsus, and tarsometatarsus, when the leg length is considered as the whole, are shown in table 6. The values of  $\log a$  (mean logarithms of leg length) for these regression equations remain the same as for the body weight and are shown in table 5. The three values of  $k$  show a significant trend within each of the

TABLE 6  
VALUES OF  $k$  FOR LENGTH OF LEG BONES WITH RESPECT TO TOTAL LENGTH OF LEG

Mating	Group	Femur*	Tibiotarsus*	Tarsometatarsus*
Barred Plymouth Rock . . .	{ 1 2	0.925±0.095 0.940±0.053	1.025±0.010 1.028±0.010	1.041±0.077 1.027±0.167
Black Minore . . .	{ 3 4	0.915±0.010 0.918±0.116	1.058±0.335 1.026±0.097	1.031±0.010 1.032±0.157
1×4. . . . .	{ 5 6	0.925±0.045 0.945±0.010	1.024±0.045 1.021±0.045	1.028±0.045 1.020±0.094
3×2. . . . .	{ 7 8	0.938±0.010 0.944±0.063	1.021±0.045 1.010±0.071	1.050±0.032 1.046±0.010
1×6. . . . .	{ 9 10	0.952±0.063 0.976±0.010	1.006±0.010 0.999±0.010	1.037±0.084 1.045±0.010
1×8. . . . .	{ 11 12	0.907±0.045 0.937±0.071	1.021±0.063 1.020±0.010	1.063±0.010 1.040±0.010
3×6. . . . .	{ 13 14	0.925±0.010 0.913±0.675	1.011±0.063 1.014±0.010	1.076±0.010 1.061±0.114
3×8 . . . . .	{ 15 16	0.927±0.443 0.926±0.010	1.012±0.010 1.006±0.362	1.058±0.202 1.076±0.010
5×2. . . . .	{ 17 18	0.937±0.010 0.912±0.063	1.016±0.010 1.029±0.010	1.057±0.010 1.047±0.105
7×2. . . . .	{ 19 20	0.927±0.010 0.938±0.089	1.011±0.010 1.013±0.110	1.056±0.071 1.028±0.095

\* Standard errors below 0.032 are estimates.

groups, though no difference in  $k$  for the same bones in different groups can be noted. The highest  $k$  in seventeen of the twenty groups is for the tarsometatarsus while the femur exhibits the lowest value in every case without exception. That the  $k$  for tarsometatarsus is significantly higher than that for the tibiotarsus is shown by Student's method of pairing. The value of  $z$  obtained is 1.125, which for twenty pairs is equivalent to odds of over 10,000 to 1; this presents a highly significant difference. Thus the most actively growing bone is the distal one and the slowest-growing one is the proximal.

This finding can be interpreted to mean that growth gradients exist in the posterior limb of the fowl, with the center of growth-retarding influences lying at the proximal end, or conversely with the center of growth activity lying at the distal end. The effect is radiated toward the other end, so that the tarsometatarsus in the first case or the femur in the second falls under those influences to a lesser degree than the other bones. A suggestion that this may be the case was developed by Huxley (1932) on the basis of the data on rate of growth of the leg bones of chicken embryos of Schmalhausen and Stepanowa (1926). He also cites Hutt (1929) who found that castration in the White Leghorn changes the length of the leg bones to a variable degree, increasing the femur by 3.0 per cent, the tibiotarsus by 3.4 per cent, and the tarsometatarsus by 3.9 per cent. The trend of this gradation is comparable to the one found in the data herewith presented. It also parallels the trend found by Landauer (1934) and confirmed by Lerner (1936) in the reduction of bone length in Creeper fowls. Furthermore, Rulon (1935) found reduction of Janus green in the limb bud of chicken embryos to show a distal-proximal gradation.

On the other hand, Nevalomyi and Podhradsky (1930), working with two experimental birds, found that the positive effect of excess of thyroid on the leg bones was centrifugal, rather than centripetal. The growth of the femur was encouraged, the tibiotarsus was intermediate and the tarsometatarsus decreased in length. This is not necessarily a contradiction to the present findings or those of Hutt (1929) even when the small numbers involved are overlooked. The effect of thyroid extracts on genetic growth-retarding factors probably needs more elaborate investigation before such a conclusion can be made.

It may be mentioned here that Green (1933b) found evidence of gradients in the crania of mice, using methods of analysis similar to those here presented. Of considerable interest also is his finding that first-generation hybrids exhibit in many of the cases higher values of  $k$  than the parents. Green considers this a manifestation of heterosis. One (zygomatic width) out of the seven dimensions studied presented an intermediate condition between the  $k$  value of the two parents. This may be compared with Coffman's hypothesis of heterosis (1933), which presents the view that the phenomenon of hybrid vigor is specific for parts. No evidence for the existence of heterosis for any of the structures studied can be seen in the data presented in this paper. This may not hold true if the analysis were based on a time rather than on a body-weight scale. The material on hand was, however, as previously mentioned, not found suitable for such an analysis, because of the small number of birds of each sex and each group per hatch.

Table 6 also shows that the tibiotarsus and the tarsometatarsus exhibit hypergonia, while the femur shows hypogony. The net value of  $k$  for the leg length is, then, a result of positive tendencies in the case of the distal bones and a negative tendency in the case of the proximal one.

**Correlations.**—Correlations between the  $k$  values of the three bones with respect to body weight and the  $k$  for the total leg length were computed and are of a very high magnitude, owing to a certain amount of spuriousness. Thus the coefficient for the femur-leg relation is  $0.902 \pm 0.042$ ; for the tibiotarsus-leg,  $0.957 \pm 0.019$ ; and for the tarsometatarsus-leg,  $0.983 \pm 0.008$ . Evidently not the relative length of a bone, but rather its respective rate of growth, determines its contribution to the rate of the whole, as shown by the gradation of the coefficient values.

The correlation coefficients determined between the  $k$  values of the three bones are also of some interest. The femur—tarsometatarsus shows the relation of least magnitude, with the coefficient of  $0.788 \pm 0.085$ . The femur-tibiotarsus relation is next, the coefficient of correlation here equaling  $0.795 \pm 0.082$ , while the tibiotarsus-tarsometatarsus relation exhibits a coefficient of  $0.966 \pm 0.015$ . The difference is further accentuated when partial correlations are considered: the three coefficients in the same order are  $0.127 \pm 0.220$ ,  $0.213 \pm 0.214$ , and  $0.909 \pm 0.039$ . Multiple coefficients of correlation were also calculated, treating each bone in turn as the dependent variable and the other two as independent variables. A four-variable system of correlation, with the total leg length as the dependent and the three individual bones as the independent, was also set up. In all of the cases but one, the multiple coefficients fell into the range between 0.960 and 1.000. The single exception was the correlation coefficient of the femur with the other two bones, its value being  $0.801 \pm 0.080$ .

Thus it may be seen that the constant  $k$  of the femur is to a certain degree independent of the  $k$  values of the tibiotarsus and the tarsometatarsus. Wright (1932) concluded that only 8 per cent of the total variance in the femur length of White Leghorn hens may be assigned to special femoral factors, a figure closely approaching in value the 7 per cent of the variance of the tibia due to special tibial factors. If this conclusion is accepted, the correlation coefficients presented here must be interpreted to indicate a differential response of the femur to factors common to the whole leg.

There are also other points to be considered in this connection. Landauer (1934) points out that in the Creeper stock the susceptibility to growth retardation is proportionate to the time the bone is laid down during development. At the same time he finds that the greatest reduc-

tion occurs in the bone with the greatest inherent growth capacity, or having ultimately the greatest length. Under normal conditions, as here found, the bone to be laid down latest (*tarsometatarsus*) grows the fastest, eventually exceeding in length the ontogenetically oldest bone (*femur*). The longest bone (*tibiotarsus*) is laid down after the femur but grows at a more rapid rate, so that it greatly exceeds the femur in length even by the time of hatching. Its rate, however, is sufficiently close to that of the *tarsometatarsus* to maintain its relatively greater length throughout the posthatching period of growth. Thus through invoking positional effect and differential time of origin, no special factors for rate of growth need be postulated for the three leg bones. Common factors eliciting a differential response may well explain the situation.

*Sex Differences.*—With regard to the sex differences, the results of Student's method of analysis for the three bones are as follows: the *tibiotarsus* and the *tarsometatarsus* show higher *k* values for the males, the odds on significance being approximately 8½ to 1 and 19 to 1, respectively; the *femur* exhibits a higher *k* for females, with 6 to 1 odds on the significance of the difference. Since the odds are so low, none of the bones present individually a real sex difference in heterogony. Such a difference for the total leg length can be adjudged to be due to small differences in the three bones, not significant by themselves. The trend of significance is in line with the growth gradients observed.

In general, the values of table 4 and table 6 are different expressions of the same situations. The ratio of *k*<sub>1</sub> to *k* for each bone should be equal to the *k*<sub>1</sub> value of the leg length with respect to body weight. This holds true for all of the cases, any deviations being due to accumulated fourth-place decimal differences.

#### RELATION OF THE LEG LENGTH TO THE PECTORALIS MAJOR

Table 7 gives the values of the exponent in the relation

$$y = bx^k$$

1

where *y* stands for pectoralis weight and *x* for leg length. The same table also gives the values of the parameter  $\log a$  (mean values of  $\log y$ ) in accordance with equation 11. It should be noted that here again a linear measurement is equated to a mass measurement. However, contrary to what was the case when the growth of leg bones was compared to the growth in body weight, it is the linear measurement which is treated here as a whole, rather than the mass. This was dictated by practical consid-

erations, since the value of the denominator in equation 7 was already available for cases where leg length was considered as the whole.

In accordance with the equation

$$\text{Weight} = C \text{length}^k, \quad 14$$

isogony would be indicated by a  $k$  value of 3. It may be seen that the values obtained are very close to 3 and in no case show a significant deviation from it.

Applying Student's method of analysis to deviations from the value of 3, a  $z$  value of 0.322 is obtained, with the low odds of 10 to 1 on significance. It may then be concluded that the leg grows at the same rate as the pectoralis, which is a confirmation of the values of  $k$  of the two structures with respect to body weight. However, the males show slight hypogony

TABLE 7

RELATIVE GROWTH CONSTANTS FOR THE WEIGHT OF THE PECTORALIS MAJOR WITH RESPECT TO LEG LENGTH

Mating	Males			Females		
	Group	$k$	$\log a$	Group	$k$	$\log a$
Barred Plymouth Rock	1	2.074 ± 0.377	1.669	2	3.191 ± 0.385	1.482
Black Minorca	3	2.987 ± 0.347	1.513	4	3.260 ± 0.522	1.526
1 × 4	5	2.851 ± 0.344	1.408	6	3.015 ± 0.489	1.340
3 × 2	7	2.945 ± 0.326	1.382	8	2.824 ± 0.672	1.380
1 × 6	9	2.915 ± 0.366	1.178	10	3.196 ± 0.105	1.167
1 × 8	11	2.845 ± 0.338	1.438	12	3.187 ± 0.315	1.201
3 × 6	13	2.982 ± 0.446	1.060	14	2.885 ± 0.588	1.173
3 × 8	15	2.824 ± 0.509	1.112	16	2.900 ± 0.463	1.097
5 × 2	17	2.911 ± 0.506	1.311	18	2.863 ± 0.447	1.296
7 × 2	19	2.857 ± 0.541	1.261	20	2.895 ± 0.088	1.236

in all of the cases and hence a sex difference may be suspected. This sex difference is made up of a difference in favor of the males with respect to leg length and one in favor of the females with respect to pectoralis. The net result here is a small difference favoring the females, with the borderline odds on significance of 25 to 1.

The value of the coefficient of correlation between the  $k$  values for the pectoralis-major—body-weight relation and  $k$  for the leg-length—body-weight was found to be  $0.172 \pm 0.217$ . The coefficients of correlation of the same pectoralis-major value of  $k$  and the  $k$ 's for femur, tibiotarsus, and tarsometatarsus were respectively  $0.200 \pm 0.215$ ,  $0.214 \pm 0.213$ , and  $0.216 \pm 0.213$ . Obviously, none of these coefficients of correlation are significant.

As far as the lack of significance of these correlations is concerned,

two possible interpretations present themselves. One is that the growth of the pectoralis major is controlled by other factors than those governing the growth of the leg bones. The other lies in the fact that no real differences exist between any of the  $k$  values for the leg or any of the  $k$  values for the pectoralis major. Consequently it may be argued that no correlation is to be expected whether or not the factors controlling growth are the same in both structures.

### COMPARISON WITH OTHER BREEDS

The data presented in the preceding sections point very definitely to the fact that the  $k$  values for the structures investigated in the two breeds studied are the same. The fact that the various hybrids are also similar to the parental breeds in their heterogony constants would suggest that the genetic factors controlling auxano-differentiation are identical in the Plymouth Rock and the Minorca. There does not seem to be any escape from such a conclusion, and the first question which brings itself to mind is whether or not the reason for this lies in the similar adult weights of the two breeds. In order to investigate this point, an attempt was made to compare the values obtained here with breeds of other defined adult weights than that of the Plymouth Rock and the Minorca.

Unfortunately there is a distinct paucity of published material which could be used for this purpose. Measurements of bone length are available in a number of cases, but they are recorded either as averages or without corresponding body weights (Schneider and Dunn, 1924; Dunn, 1928; Hutt, 1929).

A. J. G. Maw was kind enough to supply the writer with individual records of body weights and tarsometatarsus lengths of birds used in his Brahma  $\times$  Sebright crosses. However, all of the observations were made on adult birds and because of that were found not to be amenable to the proposed analysis. Female fowls, particularly of the heavier breeds, have a tendency for a differential degree of fat deposition with increasing age. Consequently the body weights in Maw's data showed a considerable scatter when bone length was used as the second coördinate. No close correspondence was found between body weight and the length of tarsometatarsus, and no reliable  $k$  values could be calculated.

It was felt, however, that some degree of comparison could be made by using the averages presented by Maw and by other investigators. Such data were found in the report of Kopeć (1927) on Leghorns, Orpingtons, and hybrids between them; and in that of Lerner and Asmundson (1932) on Light Sussex  $\times$  Ancona hybrids. The latter workers present only measurements of tarsometatarsi of females, and hence the other

figures used were confined to that structure. In order to have comparable figures, the Plymouth Rock and Minorca females within the weight range of the adult Bantams used in this investigation (see next section) were singled out of the total population used in the present study. The logarithms of average tarsometatarsus length were then plotted against the logarithms of the average body weight. The resultant plot is presented in figure 3. This figure includes data for jungle fowl and also for Bantams,

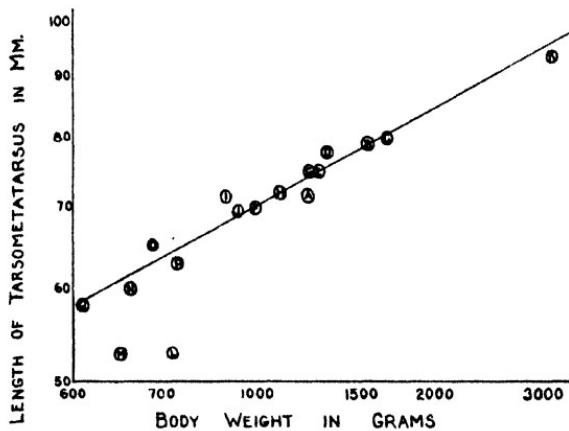


Fig. 3.—Length of the tarsometatarsus of females of different species, breeds, and crosses, plotted against body weight on a log grid. A, White Leghorn; B, Black Orpington; C, D, E, crosses of White Leghorn and Black Orpington; F, G, H, crosses of Light Sussex and Ancona; I, Black Minorca; J, Barred Plymouth Rock; K, Light Brahma; L, Bantam; M, Sebright Bantam; N, *Gallus gallus*; O, *Gallus lafayetti*; P, *Gallus sonneratii*; Q, *Gallus varius* (A to E from Kopeć, 1927; F to H, from Lerner and Asmundson, 1932; I, J, L from this paper; K from Maw, private communication; M from Maw, 1935; N to Q from Beebe, 1921.)

to which reference will be made later. The jungle fowl represented are the extant wild species of fowl, which have probably contributed to the ancestry of the domestic chickens; these are described in detail by Beebe (1921), who recorded body weights and tarsal measurements of four species, *Gallus gallus*, *G. lafayetti*, *G. sonneratii*, and *G. varius*.

The most interesting observation which can be made from this figure is that apparently the jungle fowl and all of the breeds studied except the Bantams fall into a relation which approaches a straight line. This strongly suggests that the *k* value is closely similar in these breeds. It has been shown in preceding sections that the *k* value is similar for both the pectoralis major and the leg bones in the case of the Plymouth Rocks

and the Minorcas. If this can be established for other breeds as well, it may seem that the general growth pattern is the same in the normal-sized breeds of fowl. This points to the hypothesis that a basic genetic complex for size interrelation of parts exists in the fowl, which does not necessarily have a bearing on the ultimate size. The latter may be controlled by different modifying factors. This hypothesis, however, would not include the case of the Bantams, since special conditions seem to prevail with them.

#### BANTAM DATA

*Size Inheritance in Bantams.*—In most of the work previously cited dealing with the size of the  $F_1$  generation with respect to the size of the parents in the domestic fowl the hybrid generation was found to be closer in weight to the heavier parent. Notable exceptions are seen in cases where Bantams were used as one of the parents, such as in the work of Sokolov (1926), Danforth (1929), Jull and Quinn (1931), and Maw (1935).

Sokolov suggests dominance of Bantams for size. Danforth, in crosses of Bantams with bigger breeds, obtained  $F_1$ 's either intermediate or approaching the Bantam in size. Jull and Quinn, crossing Barred Plymouth Rocks with Rose Comb Black Bantams, found the  $F_1$  generation to be closer to the Bantam weight than to that of the Plymouth Rock. Similarly, Maw (1935) in a Light Brahma  $\times$  Golden Sebright Bantam cross, found the hybrids to be smaller than the mean parental size.

Using measurements of bone length as a criterion, Maw observed that the difference in size between the two parents was due to some extent to a sex-linked factor. Not only was there a difference between reciprocal-cross females, but a study of the  $F_2$  population revealed indications of linkage of size with two of the gene markers located on the sex chromosome, which were present in his stock.

These results are of great significance, not only because they constitute the first definite confirmation of Mendelian inheritance of size in poultry, but also because they indicate the presence of a size-limiting factor in Bantams, which may be dominant in nature. It should be noted, however, that while this may explain the results of Jull and Quinn (1931), it fails to fit the evidence of Punnett and Bailey (1914), who also worked with Bantams. In their case a cross of Gold-pencilled Hamburgs was made with Silver Sebright Bantams, and the hybrid population, while intermediate in weight, approached the Hamburg more closely than the Sebright. However, the Hamburg itself is not a very large breed, and the difference between it and the Sebright is not nearly so great as between the breeds used by Jull and Quinn or by Maw. Asmundson<sup>\*</sup> found that in

\* Asmundson, V. S., private communication, January, 1936.

crosses of Leghorn males with Bantam females, the crossbred females were exactly intermediate in body weight. The cross was made only one way and it is not possible to say whether or not the sex-linked factor operated, particularly since the data on males were somewhat limited.

If the Bantams possess a major size-inhibiting factor, its influence might be shown on the relative rates of growth of parts. Since differential rates exist in the various structures, the effect of a general growth-retarding factor may be different on the different parts. Because of this

TABLE 8  
RELATIVE GROWTH CONSTANTS OF BANTAMS

Base	Part	<i>k</i> or <i>k</i> <sub>1</sub>	<i>log a</i>
With respect to body weight	Pectoralis-major weight .....	1.068±0.237	1.391
	Leg length .....	0.246±0.134	2.263
	Femur length .....	0.237±0.145	1.748
	Tibiotarsus length .....	0.231±0.145	1.889
	Tarsometatarsus length .....	0.279±0.164	1.699
With respect to leg length	Femur length .....	0.932±0.281	1.748
	Tibiotarsus length .....	0.926±0.214	1.889
	Tarsometatarsus length .....	1.111±0.297	1.699
	Pectoralis-major weight .....	3.372±1.926	1.391

situation, information on the heterogonic relation in the Bantams was thought to be of value.

*Heterogony in Bantams.*—Consequently extra series of dissections were made, for which 26 miscellaneous Bantams were available. The Bantam series comprised 23 females, mostly of the Buff Cochin breed, but with representatives of the Black Cochin, nonfrizzling segregates of a golden-laced Frizzle variety, a single-comb segregate of the White Wyandotte Bantam, and a number of unidentified types of intra-Bantam crosses. The 3 remaining individuals were males, 2 of which were also crossbred Bantams, the third being a Buff Cochin.

Dissections were performed on these birds, the same procedure as for normal-sized birds being followed. In the computations, all of the Bantams were treated together although, because of the heterogeneity of the population, great variability in the measurements taken ensued. The weight range represented was from 205 to 1,320 grams. The constants of heterogonic growth for these individuals are shown in table 8.

The first striking point that may be noted from the *k* values for the Bantams is that all of the leg bones are hypogonic, with respect to body weight, whereas in the case of the other breeds reported on (tables 3 and

4), definite hypergony was indicated. In the case of the pectoralis major, the Bantams show approximate isogony. Because of the fact that 20 of the 26 birds used for these calculations were full-grown and hence the range of true measurements was limited, the only reliable  $k$  value obtained is that for the pectoralis major. However, a possible interpretation is that the factors retarding the general growth also inhibited the growth of the pectoralis major and of the leg bones. This retardation was greater for these structures than for the whole of the body and consequently there must be some other organs in the Bantam compensating for this increased effect. It should be noted that the growth gradients in the leg observed in the Plymouth Rock and the Minorca cannot be demonstrated here. However, the high standard errors invalidate any possible deductions from this fact.

For figure 3 the averages of the 17 adult Bantam females were used. It may be seen that at least as far as the tarsometatarsus is concerned, the Bantams are distinctly out of line. This would indicate that either the Bantam has additional factors changing heterogonic relations or that this is the effect of the general growth-retarding factor. Which of the two interpretations is correct can be established by crosses of Bantams with normal-sized breeds. It is not possible to judge a priori the outcome of such a test, especially when it is remembered that Bantam breeds have undergone selection to duplicate the shape of their normal-sized counterparts. When a structure shows any condition except isogony, the arithmetic proportion of it to the whole, or the relation apparent to the eye is different at different body weights. Then selection of birds smaller in size but of the same proportions as a larger bird will lead per se to selection for differential heterogony constants. However, under the circumstances of the case on hand, since the pectoralis major grows faster than the whole of the body, one would expect that the Bantam would show increased hypergony to duplicate the final relation in the normal-sized fowl at a lower weight. As far as the tarsometatarsus is concerned, while it shows hypergony, the fact that it is a linear measure which is being compared to a mass one may possibly invalidate the reasoning.

The results presented are of interest because they have a definite bearing on theories concerning the rôle of mutations of size factors on evolution of form. The work of Robb on the equine skull may be introduced here before discussion of the application of the above data to the subject. Robb (1935) studied the development of facial proportions in a series of specimens of fossil Equidae. He concluded on the basis of his analysis that the changes in skull form observed from the Eocene *Hyracotherium* to the modern horse may be best explained on the basis of mutations

arising which affect general body size, rather than mutations affecting form as such. This would probably mean that if a general uniform morphogenetic ground plan of growth exists for chickens, any mutation affecting total body size may find an expression in the rates of growth along the various growth gradient axes.

Castle (1932c) has expressed a somewhat similar idea; he states that change in proportions may be due not to orthogenetic tendencies, but as a consequence of changed body size. The genes here are genes controlling rates; these rates may vary with the position of the structures or tissues studied in the developing organism, but, in producing a harmonious whole, they are related to each other in most of the cases. Any change affecting factors governing the sum total of these rates may find a differential expression in each constituent rate. Thus, in the case of the breeds of domestic fowl, it has been shown that a uniformity in the rate of development of the long bones of the leg and of the pectoralis major exists in the Minorca and the Plymouth Rock, yet an indication is present that the Bantams show a distinctive rate.

#### RELATIVE GROWTH AND HEREDITARY SIZE LIMITATION

The phylogeny of most of the breeds of chickens involved is lost in antiquity. Robinson (1924) states that Bantams were known from immemorial times, but that they were probably somewhat larger than at present until continuous selection by man brought them down to the present size. Aldrovandi (1637), who compiled one of the earliest complete descriptive works that included poultry, mentions Aristotle's description of the Hadrian fowls, which probably represents the earliest record of dwarf or Bantam poultry. The actual origin of these remains obscure, so that in reality no information is at hand as to the phylogenetic relation of the Bantams to normal-sized fowls.

It is quite conceivable that all of the domestic poultry originally had a uniform genetic complex controlling total body size and thus the rates of auxano-differentiation of different structures. Sometime in the phylogeny of the fowl, a basic mutation or a series of such took place and as a result of selection of the extreme mutant forms, the modern Bantam was produced. The growth patterns of the other breeds may, however, be controlled by basic factors for size limitation which are common to all of them, while modifiers may account for total size differences. In addition to these same factors, the Bantam possesses dominant or partially dominant factors which reduce its size further. This hypothesis seems to fit most of the facts of the case and can help in the interpretation of the uncertain results obtained in crosses between breeds, other than

Bantams. The difference in size between the larger and the smaller of our normal breeds may well depend on a very large number of modifiers of the basic size-limiting factors and hence the  $F_2$  population required to demonstrate this would probably be above the limits of practical experimentation.

An interesting parallel to these ideas lies in the study of evolutionary relative growth in the titanotheres by Hersh (1934). He has found that various heterogonic constants of skeletal structures of a number of Eocene and Oligocene genera of titanotheres differed from each other. At the same time ascending species within the genus showed no change in either  $b$  or  $k$ . The evolution of titanotheres in size is interpreted by Hersh as a result of incorporation in the stock of mutant genes bringing about an increased number of cell divisions. The coefficient of heterogonic growth was the factor indicating the partition of this greater number of divisions in different directions, and since it differed in various organs and structures, a change in proportions followed.

It is evident that a similar line of reasoning may be applied to the gallinaceous species. However, the case of the Bantam, which is classified as belonging to the species *Gallus domesticus* together with the other breeds, affords an extension of the principle not only within a genus, but within a species. A change in the heterogony constants is suggested here, and since by no stretch of imagination can the Bantam be placed as a taxonomically separate species from other domesticated breeds of poultry (though in nature morphological isolation would exist between Bantams and, for instance, Brahma, because of the disparity in size), it must be concluded that differences in heterogony constants may be present in coexisting varieties of one and the same species.

Additional evidence for a difference in the genetic complex of factors controlling growth and development between Bantams and other breeds is found in the work of Promptoff (1928). In studying structural types of the dorsosacrum he reported that most of the breeds are possessed of four or five dorsosacral vertebrae, but that the Bantam has only three. The mode of inheritance of the character was not clearly described by Promptoff, since only a small number of individuals were available. However, a general growth-retarding factor may be easily conceived to be responsible for the difference found. A parallel suggestion is made by Sawin (1935) to account for the variability in number of ribs and vertebrae in the skeleton of the rabbit.

The time of action of the growth-retarding factor can be estimated in the ontogeny of the Bantam to lie at the time of active histo-differentiation of the dorsosacrum, which is concurrent with differentiation of the

posterior limb bud. It may once more be recalled that Danforth (1932) produced rumpless fowls by fluctuating incubator temperature in the first week of incubation. A hereditary factor may be involved with a parallel effect in the case of the Bantam. It is, of course, quite possible that the mutation involved is one of the type described by Haldane (1932), which affects the time of action of a gene. Changes in the value of  $b$  would particularly fall into this class, though  $k$  values may also be affected. Stockard's interpretation of the genetic bases for achondroplasia (1932) may be viewed in the same light.

An interesting case of a change in the value of  $b$  without a change in  $k$  is found in chubby *Drosophila*. Dobzhansky and Duncan (1933) plotted body length against body width on a double logarithmic scale for the larvae of wild-type *Drosophila* and of the mutant chubby type, which exhibits a different shape than the normal. They found the slope to be the same in the two instances but a distinct difference may be noted in the intercept on the plot they present. Here, the mutation exhibited in an early developmental stage does not affect the rate of a process, but its time of onset. Such interpretation approaches Hersh's (1934) application of Brandt's (1928) "Typologisches Grundprinzip" to his discussion of the case of the titanotheres.

A question of interest is brought up by Kaiser (1935) in his study of factors governing shape and size in the fruits of peppers. He extends the concept of Ford and Huxley (1927), who conclude that a defined rate of relative growth is inherited, by stating that developmental genes govern not merely growth velocities but the relation between these velocities. This is somewhat similar to Sinnott's conception of the size and shape differences in gourds (1935c). Here the smaller race is essentially like an early developmental stage of the larger one, the shape difference being due to the effect of size.

Parallel reasoning cannot be applied successfully to the instance of the fowl at this time. It is true that in the different normal-sized breeds, which in their zygotic state carry factors defining ultimate weight, similar factors for the relative rate of growth of both the pectoralis major and the leg bones are present. In the case of the Bantam the proportionate growth rates of parts are changed concurrently with the change in size. Yet the Bantam, as shown in figure 3, is obviously not representative of the earlier stages of growth of the normal-sized breeds.

The test of Kaiser's dictum regarding the interrelation of the growth rates of parts would come in the crosses of Bantams with the larger breeds. Such crosses should be of considerable interest and should yield valuable information on this and other points. A limited number of ob-

servations are already available and further work along these lines will be undertaken.

### SUMMARY

Investigations of heterogonic growth relations reveal that the growth of the pectoralis major of the leg bones in relation to the growth of the entire organism follows a similar course in the Plymouth Rock and the Minorca breeds of fowl. Hybrids between these two breeds including first-generation crosses as well as backcrosses of these to both parents, possess the same pattern of growth of the structures studied as the parents. Sex differences are noted in the values of the coefficient of heterogonic growth, with the females showing a higher value for the pectoralis major with respect to body weight and a lower value for the leg length with respect to body weight than the males.

Comparison of the data presented with the material of other investigators suggests the hypothesis that the basic genetic complex for the type of growth of parts with respect to the growth of the whole is common to the different breeds of poultry with the exception of the Bantams. The Bantams, a number of which are also included in this study, show a type of heterogony possibly different from that observed in the larger-sized breeds. While in the latter hypergony was indicated both for the muscle investigated and for the leg bones, in the Bantam hypogony was present in the leg bones, with an approximation to isogony in the muscle. It is thus suggested that the Bantams probably possess growth-retarding factors which affect not only total body size but also the relative rate of growth of the parts studied.

Growth gradients based on length measurements were observed in the posterior limb, with the distal bone (tarsometatarsus) growing at a more rapid rate in relation to the rate of body growth, the median bone (tibiotarsus) presenting an intermediate rate of growth, and the proximal bone (femur) the slowest.

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THE CONNECTION BETWEEN DEMATOPHORA  
NECATRIX AND ROSELLINIA NECATRIX

H. N. HANSEN, HAROLD E. THOMAS, AND H. EARL THOMAS

A BACTERIAL GALL DISEASE OF DOUGLAS FIR,  
PSEUDOTSUGA TAXIFOLIA

H. N. HANSEN AND RALPH E. SMITH

APPLE MOSAIC

H. EARL THOMAS



# H I L G A R D I A

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## THE CONNECTION BETWEEN DEMATOPHORA NECATRIX AND ROSELLINIA NECATRIX<sup>1,2</sup>

H. N. HANSEN,<sup>3</sup> HAROLD E. THOMAS,<sup>4</sup> AND H. EARL THOMAS<sup>5</sup>

SINCE THE FIRST THOROUGH STUDY of *Dematophora necatrix* (Hart.) by Hartig,<sup>(1)</sup> who suggested its relationship to the genus *Rosellinia*, there has been a reasonable doubt as to the reality of that relationship in spite of the finding of an associated *Rosellinia* stage by Viala<sup>(2)</sup> and later by Prillieux.<sup>(3)</sup> The reason for this doubt is made clear by Viala, who says:

"Nous avons essayé, par tous les precedes, d'obtinir la germination de ces sporides sans jamais pouvoir y parvenir. La démonstration expérimentale de la relation des périthèces et des autres formes du *D. necatrix* manque donc."<sup>(2)</sup> (p. 82.)

### PRODUCTION OF PERITHECIA

Recently,<sup>(4)</sup> we reported on the occurrence of a highly destructive fungus on apple roots in California that so precisely resembled *Dematophora necatrix*, according to Hartig's description, that we did not hesitate to name it such. From time to time since late 1933, we have collected roots from apple trees killed by this fungus and kept such material in containers under various environmental conditions in an effort to produce the ascigerous stage reported by previous workers. Late in 1935, almost two years to a day after collecting the first material, mature perithecia were observed on four pieces of root that had been kept in moist cham-

<sup>1</sup> Received for publication December 16, 1936.

<sup>2</sup> We wish to express our appreciation to Mr. S. F. Ashby, Director of the Imperial Mycological Institute, and to Mr. E. W. Mason, of the same institution, for sending specimens of *Rosellinia arcuata* and *R. buxi*, and for other courtesies rendered.

<sup>3</sup> Assistant Professor of Plant Pathology and Assistant Plant Pathologist in the Experiment Station.

<sup>4</sup> Assistant Plant Pathologist in the Experiment Station.

<sup>5</sup> Associate Plant Pathologist in the Experiment Station.

<sup>6</sup> Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

bers in the laboratory. In appearance, these perithecia (plate 1, A, B, E) are typical of the genus *Rosellinia* and agree closely with the descriptions of those studied by Viala<sup>(6)</sup> and by Prillieux.<sup>(7)</sup>

These investigators describe the perithecia as lacking ostioles, whereas in our fungus a definite round pore, or ostiole, is found at the apex of the papilla. The pore is not discernible in old perithecia that have ceased to discharge spores; for in such, the opening is filled with the dried gelatinous material in which the spores are exuded. The structure is readily demonstrated in young perithecia. The ostiole is illustrated by Hartig<sup>(8)</sup> in *R. quercina*, and we have observed it in *R. aquila* (Fr.) De Not., *R. linderae* Pk., *R. arcuata* Petch., and *R. buxi* H. Fabre.

In spore measurements, also, there is a slight discrepancy: Viala<sup>(6)</sup> gives the mean size of ascospores as  $40 \times 7\mu$ , Prillieux the range  $43$  to  $47.5 \times 7\mu$ , whereas our measurements, based on 300 spores are: range  $31.1$  to  $47.6 \times 5.1$  to  $7.1\mu$ , average  $37.1 \times 6.3\mu$ . We do not, however, consider these differences to be significant. From general observations on size ranges in spores of other species of *Rosellinia*, and for that matter in spores of most fungi, Prillieux's range seems unduly small, which suggests that it was probably based on the measurement of very few spores.

In general, the ascospores are as described by Viala,<sup>(6)</sup> including the hyaline episporule. This is readily seen in unstained material and very noticeable during early stages of germination, when it often becomes greatly distended just before the germ tubes break through (plate 1, C). The spore is typically dorsi-ventral; and in the middle of the ventral side, a slit or suture is seen running parallel to the long axis of the spore and about one-third its length (plate 1, D). This slit is not mentioned in the literature as occurring in *Rosellinia necatrix* (Hart.) Berl. nor does Massee<sup>(9)</sup> mention its presence in *R. radiciperda* Mass. Hartig<sup>(8)</sup> shows it in *R. quercina* Hart., and we have observed it in the five species of *Rosellinia* examined by us.

#### GERMINATION OF SPORES, AND PATHOGENICITY

The abundant production of conidia in *Rosellinia necatrix* would lead one to suspect this spore form to be the principal agent of dispersal. Viala<sup>(6)</sup> reports ready germination of conidia, whereas Hartig<sup>(8)</sup> made many attempts but succeeded in only one; and in that case the culture was lost before it could be adequately studied. Though we used a large number of media and treated the conidia in various ways to stimulate germination, all our efforts resulted negatively.

At first we had difficulty with the ascospores also, but germination was finally obtained by the following method: Spores were suspended in 2 cc.

of 5 per cent lactic acid. After standing for 15 minutes, 10 cc of water was added to reduce the concentration of acid, and the mixture was then poured over the surface of hard potato-dextrose agar (3 per cent agar) in petri dishes at the rate of about 1 cc per dish and incubated at room temperature (22–24° C).

Only about 3 per cent of the spores germinated, and all of those within 24 hours. Subsequent germination tests all gave the same small percentage, with no additional spores germinating after 24 hours. Germination in all cases was through the ventral slit or suture (plate 1, G).

Fifty of the germinated spores were transferred singly to potato-dextrose agar, where they continued to grow and eventually (within 24 days) produced the coremial stage of *Dematophora necatrix*. Some of these cultures were used to inoculate eight young apple trees by placing the inoculum in contact with their roots. Within six weeks, all the inoculated trees were dead, whereas the controls remained healthy.

The germination of ascospores, with subsequent production in culture of the coremial stage and the demonstration of pathogenicity, are considered to constitute adequate proof of the genetic relation of *Dematophora necatrix* to *Rosellinia necatrix*. Specimens bearing perithecia have been sent to the Imperial Mycological Institute at Kew and the New York Botanical Gardens.

#### ASSOCIATED FUNGI

Viala<sup>(9)</sup> describes in detail a pyrenidial fungus which he considers to be a stage in the life cycle of *Rosellinia necatrix*. Massee<sup>(10)</sup> also describes a pyrenidial stage in *R. radiciperda*, and Hartig<sup>(11)</sup> found pyrenidia associated with *R. quercina* but states that he was unable to prove the relationship.

We have found constantly associated with *Rosellinia necatrix*, on apple roots, a pyrenidial fungus, which upon culture proved to be a species of the form genus *Phomopsis* and in no way related to the true pathogene. The constancy of its presence, however, might easily lead one astray unless culturing is resorted to.

In apple orchards where the root rot is prevalent, and also in orchards where it has not yet been observed, we find another species of *Rosellinia*, tentatively identified as *R. aquila*. This fungus fruits abundantly on old apple prunings left in the orchards from year to year. It is, however, readily distinguished from *R. necatrix*, even in the field, because of its distinct conidial (*Sporotrichum*) stage. In the laboratory, the marked difference in size and shape of ascospores makes differentiation a routine matter.

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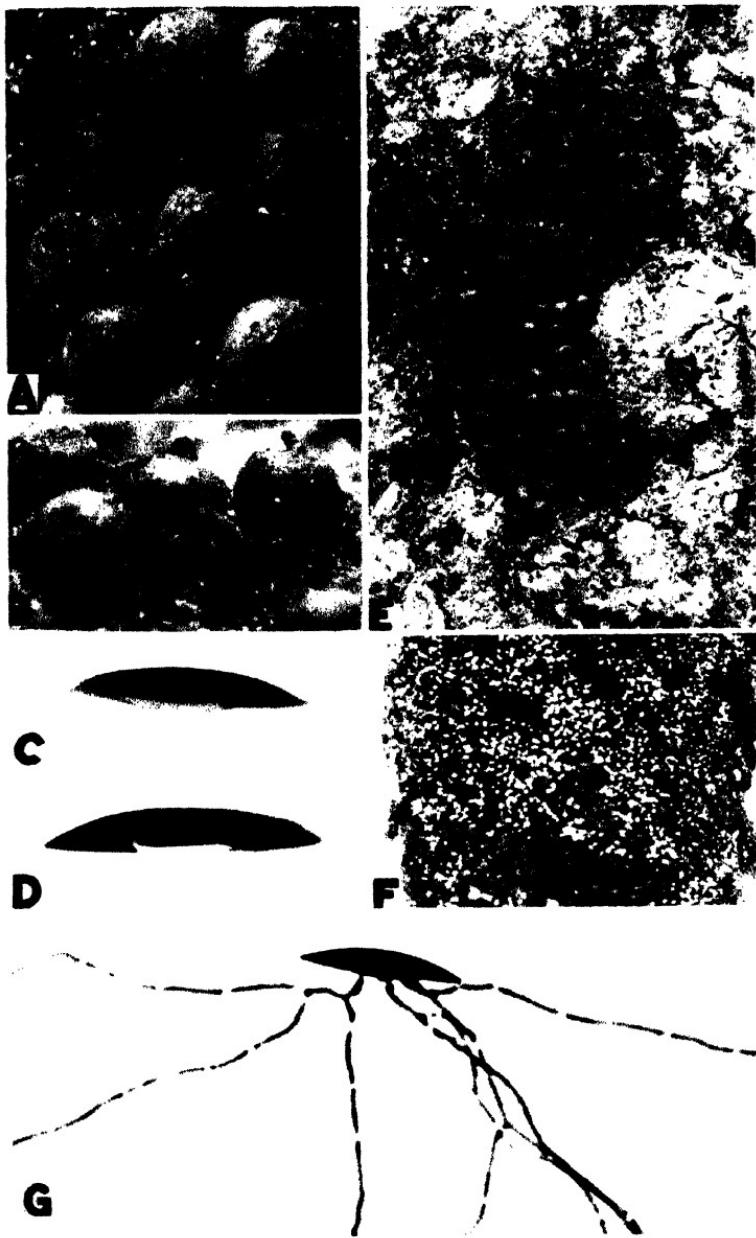


Plate 1.—*Rosellinia necatrix*: *A* and *B*, perithecia showing freshly exuded spore masses ( $\times 7$ ); *C*, spore, showing the distended episporium just prior to germination ( $\times 900$ ); *D*, spore, showing the ventral slit partly broken ( $\times 900$ ); *E*, perithecia on apple root ( $\times 1.5$ ); *F*, *Dermatophora* or coremia stage on apple root ( $\times 1$ ); *G*, germinating ascospore ( $\times 500$ ).



A BACTERIAL GALL DISEASE OF DOUGLAS FIR,  
*PSEUDOTSUGA TAXIFOLIA*

H. N. HANSEN AND RALPH E. SMITH



# A BACTERIAL GALL DISEASE OF DOUGLAS FIR, PSEUDOTSUGA TAXIFOLIA<sup>1</sup>

H. N. HANSEN<sup>2</sup> AND RALPH E. SMITH<sup>3</sup>

BACTERIAL DISEASES of conifers are exceptionally rare; as far as we know only two have been reported in the literature to occur naturally on twigs, branches, and upper stems of members of the family *Pinaceae*, and both of these are probably produced by the same organism.

In 1888 Vuillemin,<sup>(3)</sup><sup>4</sup> isolated an organism from galls occurring on twigs of *Pinus halepensis* Mill. and named it *Bacterium pini* Vuill. In 1911 Von Tubeuf<sup>(2)</sup> isolated what he considered to be the same organism from galls on twigs and branches of *Pinus cembra* L. Several attempts were made by Vuillemin and by later investigators to produce the disease by inoculating with pure cultures of *B. pini* and by transfer of gall material from diseased to healthy plants, but in no case were positive results obtained.

In 1933 Hansen and Smith<sup>(1)</sup> published a brief note recording the finding of bacterial galls on Douglas fir (*Pseudotsuga taxifolia* Britt.) in California. The present paper reports additional studies of this disease, its transmission, and the pathogen involved.

## ECONOMIC IMPORTANCE

The disease has been observed commonly in parts of Napa, Lake, Santa Cruz, Amador, and Siskiyou counties in California, in marginal localities for the growth of Douglas fir, which here occurs in mixed stands composed of several species of conifers and three or four species of broad-leaved trees. These marginal localities, though practically worthless for timber production, are much used for recreational areas, sanitariums, summer resorts, and private summer homes. As far as the value of such places may be materially lowered by the presence of dead, dying, and deformed trees, the disease can be considered to be of economic importance. In its present known range, the Douglas-fir gall disease is otherwise of no economic importance, and of only potential interest to the lumbering industries. Should it invade areas where Douglas fir is now the predominating species, it might very readily become an important

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<sup>4</sup> Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

factor in determining the future composition of the forest. If the reproduction of a single member of the stand is attacked, associated species would gain a natural advantage, and they should increase numerically in direct proportion to the havoc wrought by the disease.

#### DESCRIPTION OF THE DISEASE

The disease is characterized by the occurrence of galls on twigs, branches, and upper stems of the host. New galls are formed only on the younger

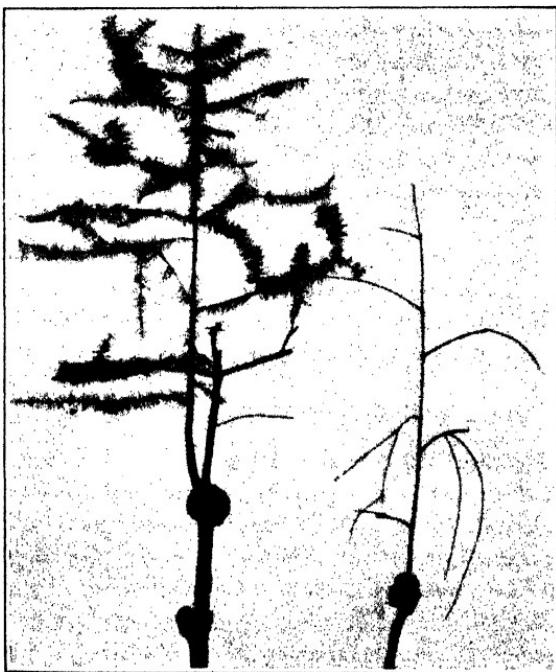


Fig. 1.—Douglas-fir trees girdled and partly girdled by bacterial galls. ( $\times \frac{1}{10}$ .)

trees up to about fifteen years of age, and most frequently on very young trees growing in crowded stands in rather damp situations near streams, ponds, or swamps. The galls most often occur on twigs or small branches but not uncommonly also on the main stems, where they occasionally completely girdle a tree, and thus give rise to an unsightly dead top commonly referred to as "spike top" or, if a secondary leader is formed, it is called a "stag head" (fig. 1). Most of the trees handicapped by having one or more galls on the main stem usually die within a few

years because they are unable to compete successfully with healthy trees. Occasionally an isolated tree with stem galls will live for several years and may even reach small timber size; but since the galls also continue to grow, the trunk is usually so badly deformed that it is useless for anything but firewood (fig. 2). The health of the host does not appear to be

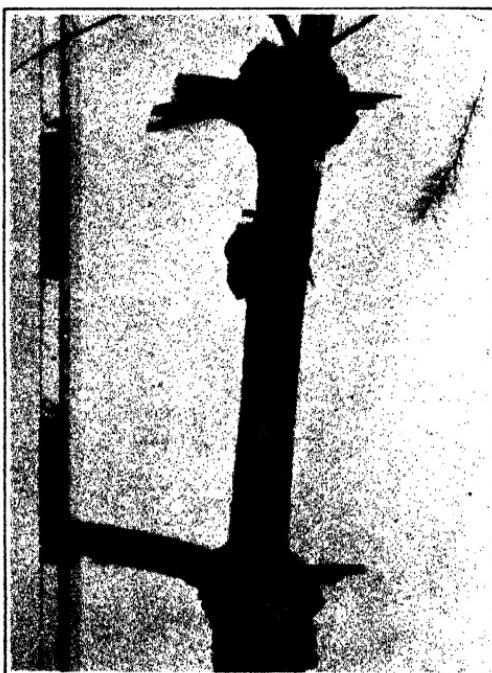


Fig. 2.—Part of Douglas-fir stem, showing old galls. ( $\times \frac{1}{10}$ .)

seriously threatened by infected twigs since these are gradually shaded out and dropped as the tree grows older.

The galls vary in size from that of a pinhead to several inches in diameter. They are globular in shape, with a rough, spongy, fissured surface which breaks out in a typical more or less cross-shaped pattern (fig. 3, A to D). During the first year, they are much lighter in color than the bark of the host and therefore stand out rather prominently. In older galls, the shape is materially altered and the typical surface markings gradually disappear. These changes in appearance can probably be attributed to insects and saprophytic fungi, which invade practically all galls that are more than one year old.



Fig. 3.—*A*, Natural galls showing typical cross-shaped markings ( $\times 1\frac{1}{2}$ ); *B*, *C*, and *D*, galls produced by experimental inoculation ( $\times 1$ ); *E*, section of natural gall, with arrow indicating point of origin ( $\times 1$ ); *F*, twig of Douglas fir from which bark has been stripped to show wounds caused by *Chermes cooleyi* ( $\times 1$ ).

The gall is composed of hypertrophied tissues, involving both stele and cortex, and is very similar in structure to that of the olive-tree galls produced by the bacterial pathogene *Bacterium savastanoi* E.F.S. There is this important difference, however, that the olive-tree gall can be produced in cortical tissues without involving any of the xylem elements,

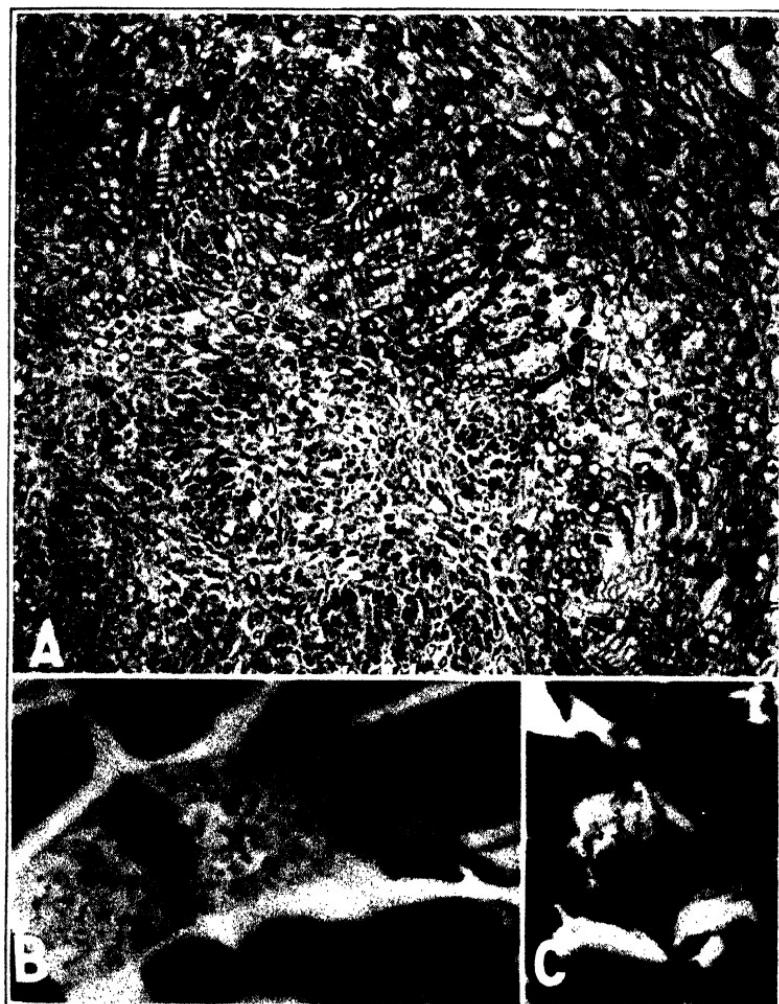


Fig. 4.—A, Section of a gall showing groups of rapidly dividing cells and also the presence of woody elements among these groups; B and C, photomicrograph of *Bacterium pseudotsugae* n. sp. in intercellular spaces.

whereas in all the Douglas-fir galls examined, both those occurring naturally and those produced experimentally, the inner core was found to be always woody in nature, and furthermore the point of origin of the galls was always found to be located within the stele (fig. 3, E). The internal structure of the galls is characterized by the presence of many more or less spherical groups of rapidly dividing cells, near or in the

centers of which occur comparatively large intercellular spaces which contain the pathogene (fig. 4, *B* and *C*).

#### PATHOGENICITY OF THE CAUSAL ORGANISM

Douglas firs were inoculated for the first time on May 15, 1932. Bacteria from pure 48-hour-old cultures were smeared on freshly made leaf scars on previous season's growth and inoculated into the cortex in shallow needle stabs and into the wood in deep needle stabs. Observations were made at various intervals during summer and fall, but all the stab wounds, inoculated and controls, appeared to have healed over rapidly and all looked alike. Additional inoculations by the same methods were made on the fifteenth of January, February, March, and April of 1933.

When the January inoculations were examined on April 15, small galls varying from 1 to nearly 5 mm in diameter were found on the inoculated deep-stab wounds. None of the leaf-scar or shallow-cortex wounds showed any signs of gall formation. Upon examination of the trees inoculated in May of the previous year, it was found that all of the deep-stab inoculations had developed small galls and that inoculated leaf scars and shallow stabs had not.

During the summer of 1933, galls developed on all deep-stab inoculations made in January, February, and March but on only one of those made in April. The remainder of these, however, began to show small galls early in April of 1934. This clearly shows that the pathogene can be present in the host for nearly a year before definite symptoms (galls) begin to appear. It is also fairly evident that gall formation and gall growth is limited to the active growth period of the host, which is roughly from late March to July in Berkeley, California, where the experimental work was done.

The causal organism was reisolated from experimentally produced galls and used for inoculations made in August, 1933, and in January, 1934. Of the 10 wounds inoculated in August, only 3 developed galls. This indicates that the organism finds it relatively difficult to establish itself during late summer, perhaps because of the greatly reduced growth rate of the host at that time of the year. The 8 wounds inoculated with the reisolate in January produced galls during the spring of 1934.

#### TRANSMISSION

Actual contact of the pathogene with xylem elements of the host tissues appears to be essential to gall formation, on evidence of the facts that the point of origin of all galls examined was found to be located in the

stele and that no galls were produced in inoculated leaf scars or in shallow-cortex wounds. These facts would seem to eliminate water, the principal agent of dispersal of the organism causing olive knot, as a carrier, and indicate that insects capable of producing rather deep wounds were responsible for transmission.

Wherever we have found the Douglas-fir gall disease, two insects—the orchard cicada, *Platypedia arcolata* Uhler.; and Cooley's chermes, *Chermes cooleyi* Gill.—have been present in spring and early summer. The cicada oviposits in twigs of the Douglas fir and in doing so causes deep wounds through which splinters of wood protrude. The egg cases remain within the wood and can be found there several years after the wound has healed. More than 200 cicada wounds from one to four years old have been examined, however, and in no case was there any evidence of gall formation.

The other possible carrier, *Chermes cooleyi*, is a sucking insect that feeds on the juices of young Douglas-fir twigs. In these it produces deep feeding punctures that penetrate through the bark and cortex into the wood. Figure 3, F, shows a twig from which the bark has been stripped to show the typical wounds produced by the insect. In the middle of each of the transverse lines is a small hole penetrating into the wood to a depth of about a millimeter. In the early spring of 1934, all our experimental trees were heavily infested with these insects, and they were observed to feed on some of the experimentally produced galls. In August of the same year, we found three galls which were definitely traced to wounds produced by *Chermes cooleyi*. Von Tubeuf<sup>(2)</sup> suggests that a species of *Chermes* is probably responsible for the spread of *Bacterium pini*.

In the localities where the Douglas-fir gall disease occurs, we find the host associated with the following conifers: *Pinus lambertiana* Dougl. (sugar pine), *P. monticola* Don. (silver pine), *P. ponderosa* Dougl. (ponderosa pine), *Libocedrus decurrens* Torr. (incense cedar), and *Abies concolor* Lindl. and Gord. (white fir). We have never found the disease on any of the above species though branches of some of them were occasionally found intermingled with those of infected Douglas fir. As further evidence that the pathogene is highly specific, it was inoculated into the following plants with negative results: *Pinus halepensis* Mill., *P. lambertiana* Dougl., *P. radiata* Don. (Monterey pine), *Tsuga heterophylla* (Raf.) Sarg. (coast hemlock); and into the following herbaceous plants frequently used to test the pathogenicity of the crown-gall organism, *Pseudomonas tumefaciens* Town.: tomato, begonia, beans, and *bryophyllum*.

## TAXONOMY OF THE CAUSAL ORGANISM

On cultural, morphological, and physiological bases, the causal organism appears to be distinct from previously described plant pathogens and should therefore be considered a new species. Hence, we suggest the name *Bacterium pseudotsugae*.

TECHNICAL DESCRIPTION OF BACTERIUM  
PSEUDOTSUGAE N. SP.<sup>6</sup>

A nonmotile rod with rounded ends, averaging in size  $1.9-3.9 \times 0.5-1.5\mu$ ; frequently occurring in pairs; non-spore-forming; Gram-negative, non-acid-fast; stains readily with analine dyes; facultative aerobe; liquefies gelatin; slight H<sub>2</sub>S produced; nitrates reduced; no acid in milk; no ammonia produced; starch not hydrolyzed; no acid and no gas produced in lactose, sucrose, or glycerine; acid but no gas produced in glucose, levulose, galactose, and maltose. *On nutrient agar slant*, growth scanty, flat, glistening, smooth-surfaced; translucent whitish; medium unchanged. *On potato dextrose agar slant*, growth moderate, slightly spreading, with wavy margin, slightly raised, glistening; surface somewhat contoured; whitish, translucent, becoming brownish with age; medium unchanged. *On potato dextrose peptone agar slant*, growth abundant, spreading, with irregular margin, flat, glistening, becoming dull with age; surface contoured; grayish white; medium unchanged. *On potato cylinder*, growth moderate, spreading, viscid, white becoming brown with age; medium turns brown. *In nutrient broth*, growth slight, no surface growth, clouding slight, no sediment. *In potato dextrose peptone broth*, growth abundant; partial ring formed; clouding strong; sediment fairly abundant; flocculent. *In S. A. B. broth*, growth moderate, no surface growth; clouding slight; sediment scanty-viscid. *In Fermi's solution*, growth moderate, no surface growth; clouding slight to moderate; no sediment. *In Conn's solution*, no growth. *In Uschinsky's solution*, no growth.

<sup>6</sup> We are indebted to Mr. George Zentmyer for testing the physiological reactions of the pathogene.

## SUMMARY

A gall disease of the twigs and stems of Douglas fir (*Pseudotsuga taxifolia*), is described and shown to be of bacterial origin. It is suggested that the causal organism is insect-transmitted, the carrier being probably *Chermes cooleyi*. Three species of pine, one of hemlock, and several herbaceous plants were inoculated with negative results. A technical description is given of the causal organism, which is named *Bacterium pseudotsugae* n. sp.

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# **APPLE MOSAIC**

**H. EARL THOMAS**



# APPLE MOSAIC<sup>1</sup>

H. EARL THOMAS<sup>2</sup>

AN INFECTIOUS VARIEGATION of apple foliage seems to have been recognized by Vibert in France as early as 1835.<sup>3</sup> Earlier reports from the northeastern United States recently reviewed<sup>4, 5</sup> indicate that mosaic of apple is general in that area, though seldom if ever destructive. Mottled apple foliage has been noted in the State of Washington,<sup>6, 7</sup> some of which may represent the disease under consideration here. A mosaic type of disease which is probably distinct has been found on an ornamental apple in Kentucky.<sup>8</sup> A report<sup>9</sup> of apple mosaic has appeared from Bulgaria, but the illustrations accompanying it are more characteristic of noninfectious types of chlorosis.

Specimens of mosaic in the variety Ranier were received from Paradise, California, in June, 1932. The trees had been purchased from a nursery in the State of Washington about five years earlier. In August, 1936, a single tree of Smith Cider obtained from a local nursery was found affected in a garden at Berkeley. Some of the affected trees or branches in the orchard at Paradise show, in addition to the direct loss of functional leaf area, a sparseness of foliage and reduction of terminal growth which seems to be chargeable to the mosaic disease.

## PLANTS AFFECTED AND SYMPTOMS

Heretofore the disease has apparently been known only on the cultivated apple, *Pyrus malus*. On this plant, the typical symptoms have been amply illustrated<sup>10, 11</sup> (fig. 1). In addition to the symptoms commonly seen on the apple, there occasionally appears a complete chlorosis of the larger veins (vein clearing) while the remainder of the leaf retains the normal form and color.<sup>10</sup> Of particular interest is the tendency of the chlorotic areas to be entirely killed both at Paradise and Berkeley during the summer months of intense sunlight.

In addition to the varieties Ranier, Smith Cider, and Starking, on which natural infection has been seen in California, all of the following developed symptoms when inoculated by grafting: Golden Delicious, Gravenstein, Lady, Tompkins King, White Pearmain, and Yellow New-

<sup>1</sup> Received for publication December 16, 1936.

<sup>2</sup> Associate Plant Pathologist in the Experiment Station.

<sup>3</sup> Reported by Bradford and Joley.<sup>10</sup> Original not seen by the writer.

<sup>4</sup> Superscript numbers in parentheses refer to "Literature Cited" at the end of this paper.

town. Several other varieties, including Yellow Bellflower are already on record<sup>"</sup> as susceptible.

Since a rather large number of the relatives of the apple are grown in California for fruit or ornament, several experiments were made to determine whether some of these might be susceptible to the mosaic of apple. Inoculations were made by grafting buds, scions, or inarches

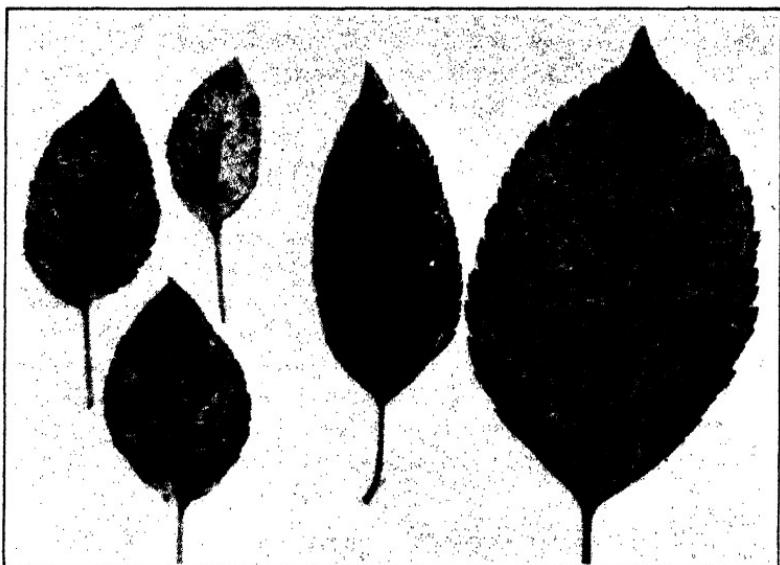


Fig. 1.—The two larger leaves at the right (White Astrachan) are affected by mosaic. The three smaller leaves (Esopus Spitzenberg) are taken from a case of genetic variegation.

from affected to healthy potted plants, or by grafting healthy scions on diseased plants. These tests were on a small scale and involved in some cases only one or two plants. The negative results are therefore not always conclusive.

*Cotoneaster harroviana* became affected after inoculation by inarching, with the production of pale bands and rings in the leaf blade. The symptoms were inconspicuous, but the virus was recovered without apparent loss of virulence by inoculation from this species to apple and rose.

The loquat, *Eriobotrya japonica*, developed strong chlorotic symptoms resembling those on the apple and in addition, in young leaves, a considerable amount of necrosis along the larger veins, resulting in marked distortion of some leaves (fig. 2). This necrosis developed under glass

and was not preceded by any marked chlorosis as is the case with the apple. The virus was recovered from loquat by inoculation to rose.

Three of 5 plants of toyon (*Photinia arbutifolia*) developed symptoms during the year following inoculation. The chlorotic spots which resulted were similar to those on the apple but few in number.

Although it is difficult to obtain a graft union between the rose and



Fig. 2.—Mottling and leaf distortion of loquat produced by inoculation with the apple-mosaic virus.

members of the pome group, definite symptoms were obtained on 5 of 8 plants of Independence Day and Belle of Portugal roses which were inoculated by inarching with affected apple, *Cotoneaster*, or loquat (fig. 3). The virus was recovered from one of these roses by inoculation to apple. The symptoms vary appreciably and may include vein clearing on occasional leaves, but usually approach the type seen on the leaf at left in figure 3. From the type of symptom and the rate of development in the plant, it is inferred that this disease is distinct from the several virus diseases that have been found occurring naturally on the rose in central California.

A single plant of *Sorbus pallens* inoculated by a scion of affected apple, developed rather strong symptoms resembling those on the apple.

Attempts to cause infection in *Amelanchier alnifolia* (western service berry), *Crataegus douglasii* (western black haw), *Cydonia oblonga*

(quince), *Pyracantha gibbsii yunnanensis* (yunnan fire thorn), *Pyrus communis* (pear—Bartlett variety), and *Sorbus sitchensis* (western mountain ash) have failed, as have also the attempts to recover the virus from inoculated plants of Amelanchier, Crataegus and pear. Recovery was not attempted with the others in this group.

Thus far the discussion has dealt with what is presumed to be a single disease. Other types of chlorosis are not infrequently found on apple

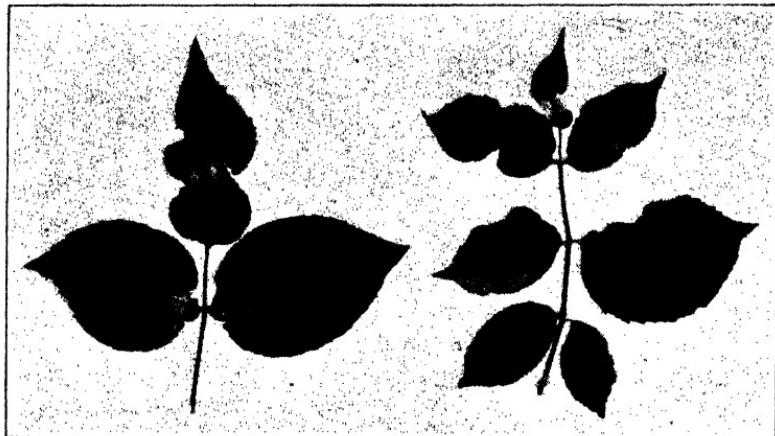


Fig. 3.—Symptoms on Belle of Portugal rose produced by the apple-mosaic virus.

foliage, some of which are not readily separated by symptoms alone from the typical mosaic. Of five such cases encountered without special search in a three-year period, three seemed to be definitely genetic in origin. One of these (fig. 1) was propagated at Berkeley and gave no evidence of transmission by grafting to healthy apple. In another case, a single orchard tree of the Tompkins King variety bore, on leaves scattered generally through the tree, symptoms that were not distinguished from those of the common mosaic. Scions from this tree were grown at Berkeley during the season of 1935 and additional ones in 1936. None of these developed any symptoms except two which were inoculated by inarching with known mosaic apple shoots. Three of the Tompkins King scions, after growing for one season at Berkeley, were top-worked with healthy scions of the susceptible Golden Delicious variety. The latter also remained free of symptoms except one inoculated as above with known apple mosaic. These results indicate that the agent which produces symptoms in the Tompkins King tree is distinct from the common mosaic virus and does not induce resistance to the latter.

A tree of the kaido crab (*Pyrus micromalus*), growing in a garden at Berkeley, bore symptoms in 1933 suggestive of those figured for ornamental crab apple in Kentucky (Valleau,<sup>10</sup> fig. 25). Two seedling loquat trees that were inarched with affected kaido shoots developed mild chlorotic symptoms and in a few leaves fine necrotic lines and rings, distinct in appearance from the symptoms produced in loquat by the virus of the common mosaic. Scions from the original kaido tree were grafted on a White Astrachan tree in 1933 but no symptoms have developed on the latter nor on Golden Delicious, which was later grafted on the same tree (January, 1935). When scions from this Golden Delicious were transferred nine months later to a plant affected by the common apple mosaic, the resulting Delicious foliage bore typical symptoms of the apple mosaic.

#### DISSEMINATION AND MOVEMENT OF VIRUS IN THE PLANT

Blodgett, who has demonstrated the transmissibility of the disease by grafts (see Orton and Wood<sup>11</sup>) and who has had this disease under observation in orchards of western New York, reports<sup>12</sup> that during a five-year period there was little evidence of natural increase in the number of affected trees.

In the orchard at Paradise, symptoms are confined to the Ranier variety, where 22 of 53 trees were found affected in July, 1935. No new infections were found in May, 1936. None were found in several dozen trees of Delicious in the same orchard or in a block of Golden Delicious trees adjoining this orchard. The affected tree found in Berkeley was planted about fifteen years ago, yet several other trees in the same garden seemed to be entirely free from the disease. No case of spontaneous infection has been seen among the experimental plants.

While the incubation period may be as short as 53 days in apple inoculated by budding, the movement of the virus through the plant is slow, especially in the toyon and rose. When these plants were inoculated in July in one branch each about 6 inches above the base of the branch, the symptoms appeared near or above the point of inoculation the following spring but had not appeared in other branches after more than two years. One such rose plant did show symptoms on all branches after 27 months.<sup>13</sup> These and other observations show that the virus moves more slowly toward the root than upward.

<sup>10</sup> Blodgett, F. M. Personal letter, November 11, 1935.

<sup>11</sup> With one of the naturally occurring rose mosaics, symptoms have been seen (in rose) below the point of inoculation in less than 40 days from the time of inoculation.

Bearing upon the manner of dissemination are sap inoculations made on three different occasions on young leaves or stems of 12 seedlings of apple, 2 of *Pyrus baccata*, 5 of toyon, and 5 of *Cotoneaster franchetti*. Carborundum<sup>(a)</sup> was used as an abrasive on all of these. No certain symptoms were seen on any of the plants up to the time of writing, or more than 6 months after the last inoculation.

There is at the time of writing no direct evidence of dissemination by any means except by grafting. It is apparent, however, that, given an efficient natural vector, the disease is capable of affecting a considerable number of species with appreciable damage to certain of them.

### HEAT TREATMENT OF AFFECTED SCIONS

One of the most promising methods of removing virus from a plant which is propagated vegetatively seems to be exposure of affected plants or plant parts to high temperatures.<sup>(a)</sup> The expression of symptoms under varying temperature conditions (not controlled), suggests that the virus of apple mosaic has a relatively low optimum temperature.

TABLE 1  
EFFECT OF HIGH TEMPERATURES ON THE MOSAIC VIRUS IN DETACHED  
DORMANT APPLE SHOOTS

Temperature ° C	Time	Moisture	Number grafted	Number showing symptoms	Injury to scions by heating
36	7 days	Moist sphagnum	8	6	Moderate
36	11 days	Dry air*	5	0†	Severe
45	60 min.	In water	8	8	Slight
50	30 min.	Dry air	13	10	Slight
50	60 min.	Dry air	8	6	Slight

\* The basal ends of these pieces were sealed in a vessel containing water. The portions exposed to the water were discarded.

† All of the scions died within 5 weeks.

In a preliminary test in 1935, a few affected shoots of apple were exposed for 15 and 30 minutes to an air temperature of 55° C. Scions were taken from these and grafted on potted seedlings in the greenhouse. Of 3 such plants which made satisfactory growth, 1 failed to develop any symptoms during 1935. This plant did, however, bear mild symptoms toward the end of the summer of 1936. This plant was of the Gravenstein variety, which is less susceptible than some of the others tested. In the meantime in 1936, several experiments were made in which detached dormant apple shoots were exposed to different temperatures and condi-

tions of moisture and for different intervals of time. The shoots were taken from the last preceding growth cycle and were for the most part 4 to 8 millimeters in diameter. Scions from the heated shoots were grafted on potted apple seedlings and were grown in a lath-house at Berkeley.

The details of these tests and the results are summarized in table 1. The failure of certain plants to develop symptoms is probably due to early death or to insufficient growth of the scions rather than to inactivation of the virus. The expression of symptoms here, as in many other virus diseases, is related to vigorous growth of the plant. Since several of the treatments were near the limit of tolerance of the apple tissues, it seems unlikely that heating of scions will be effective in freeing them from the mosaic virus. There was no recognized evidence of attenuation of the virus in these experiments.

## SUMMARY

An apple mosaic found in California seems to be identical with the disease known in the eastern United States.

The disease has been transmitted by grafting to *Cotoneaster harrovia*, *Eriobotrya japonica* (loquat), *Photinia arbutifolia* (toyon), *Rosa* sp. (rose, Belle of Portugal and Independence Day varieties) and *Sorbus pallescens*.

Heating dormant apple shoots in several ways to near the killing point of apple tissues did not inactivate the virus.

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# H I L G A R D I A

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## FRUIT-BUD AND FLOWER FORMATION IN THE SULTANINA GRAPE<sup>1</sup>

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### INTRODUCTION

THE FRUITING HABIT of the Sultanina<sup>4</sup> grape has required the development of special cultural methods to secure satisfactory crops. These methods, however, are based solely on empirical field observations. An anatomical study of the buds should reveal the differences in fruiting habit of this variety as compared with other varieties and might lead to the development of better cultural practices. The specific objects of this study were to determine (1) the time at which fruit-bud differentiation occurs in the Sultanina, (2) the fruitfulness of the individual buds from the basal to the 20th bud, (3) the rate at which the cluster primordia develop in buds at different positions on the canes, (4) the extent of development of the cluster primordia by the end of the growing season, and (5) the sequence and rate of development of the inflorescences after growth starts in the spring.

### REVIEW OF LITERATURE

Former studies of grape-bud anatomy have dealt with the differentiation and early development of the fruit buds and to a less extent with the later stages of flower development. These studies, consisting primarily of field experiments, throw little light on the subject in question. Although Goff<sup>6,5</sup> presents considerable data on the initial stages of bud differentiation of different deciduous fruit trees, he simply states that in the grape the embryonic flower is discernible in the autumn prior to blooming. Dorsey<sup>10</sup> mentions embryonic grape clusters in the buds before opening and recognizes that each secondary division of the embryonic

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<sup>4</sup> Also called Thompson Seedless in California, and Sultana in Australia.

<sup>5</sup> Superscript numbers in parentheses refer to "Literature Cited" at end of this paper.

cluster occupies a position axillary to a bract. In his textbook Perold,<sup>12</sup> quoting from Müller-Thurgau, points out that the first cluster is initiated about the middle of June, the second cluster about July 1, and that no further initiation occurs in the buds after August 1. Partridge<sup>13</sup> places the time of fruit-bud initiation at midsummer. According to him, the primordium remains a mass of heavily nucleated cells until spring, when the cluster develops after growth starts. Snyder<sup>14</sup> shows that differentiation in the Concord begins early in June and continues in the newly forming buds throughout the growing season. Barnard<sup>15</sup> and Barnard and Thomas,<sup>16</sup> studying Sultana in Australia, discuss the problem rather extensively. Their results, however, are concerned primarily with the distribution of fruit buds on the canes and with the percentage of fruitful buds in a given location.

### METHODS USED

The materials studied were collected from the experiment vineyards of the University Farm, Davis, California. Although the soil and vines were fairly uniform, there was some variation in the size and length of individual canes on each vine. This reflected itself as variations in the sizes of the primordial clusters of buds collected on the same date and taken from the same position on different canes.

The collections of buds were made on June 7, 19, and 29; July 11 and 22; August 1 and 22; October 6; and December 5, 1933; and on March 4, 1934. A collection consists of one cane taken from each of fifteen vines. All buds of a given node were placed together and treated as one lot—fifteen buds, accordingly, for each node. All the buds on the canes for the first three collections were used. After the third collection only every other bud was taken above the 4th bud. Since, furthermore, canes of more than twenty buds are rarely retained in the pruning of this variety, no buds were taken from beyond the 20th node.

One week after the last collection of buds (that of March 4, 1934), the first buds began to open. Opening continued about fifteen days. Since the date at which growth begins changes considerably from year to year, it was thought best to include the approximate stage of development of the flower parts by average length measurements of the clusters (table 3) in addition to the date of collection. The dates of the collections of the inflorescences serve to indicate the rate of development of the floral parts. The buds were killed and fixed in Karpechenko's solution. After being washed with tap water, they were passed through alcoholic solutions of increasing concentration up to the 70 per cent solution in which they were stored until used. To facilitate penetration of the killing and fixing

solution, the hairy bud scales were removed, and the buds were put under partial vacuum. Sinking of the buds in the solution was taken to indicate satisfactory penetration.

The buds were then dehydrated with alcohol, cleared in xylene, and infiltrated with and embedded in paraffin. Since the bud scales are rather hard to cut, the embedded buds were soaked in water for one or two weeks before sectioning. A disinfectant was added to the soaking water to prevent the growth of destructive organisms. Delafield's haematoxylin was used for staining, with safranin as a counter stain.

### THE MORPHOLOGY OF THE BUD, CLUSTER, AND TENDRIL

Grape buds are generally classified as mixed buds; that is, both leaves and fruit develop from the same bud. They form in the axils of the leaves. The lower buds originate in the axil of the leaf primordia in the previous year's buds (*A* in fig. 4, plate 1, and in fig. 7, plate 2). Inside the scales covering the bud is the growing point with its leaf primordia. Each leaf has two stipules that Barnard<sup>10</sup> calls stipular scales, as large as the leaf primordium or larger. Since they lignify very early, they stain red with safranin and contain many tannin bodies. These stipular scales can be seen on both sides of the base of the petiole of the leaves near the distal end of a growing shoot, whereas only stipular scars remain beside the petioles of the older leaves toward the basal end of a shoot. The arrangement of leaf primordia is distichous. Longitudinal sections through the leaf primordia are shown in figure 4, plate 1 and figure 7, plate 2. Sections at other angles (fig. 8) show only stipular scales. The leaf is initiated as a pointed protuberance from the growing point of the bud (*L* in fig. 2, plate 1, and in figs. 7 and 12, plate 2).

The first initiation of clusters was visible the first week of June. The growing point becomes bilobed; and one of the parts as indicated at *C* in figures 1-6, plate 1, becomes the initial of a cluster, whereas the other continues to be the growing point. It is rather easy to detect whether the new differentiating apex is to be a leaf or cluster primordium, since the leaf forms from a narrow, pointed primordium (*L* in fig. 2, plate 1 and in figs. 7 and 12, plate 2) whereas a cluster of primordium is rather blunt and broad (*C* in figs. 1-6, plate 1). The cluster primordium is always opposite a leaf (*L* and *C* in fig. 2, plate 1 and in fig. 12, plate 2). Thus only sections that are cut in the plane with the growing point and the primordial cluster and leaves will show both these primordia (fig. 4, plate 1; fig. 7, plate 2; and fig. 17, plate 3). Median sections in other planes will show only stipular scales. Nonmedian sections may show clusters (fig. 8, plate 2) and stipular scales but no leaf primordia.

Snyder<sup>10</sup> states that leaf and cluster primordia are alike in the initial stages. Even in this earliest stage of differentiation, however, as figures 1 and 2 (plate 1) will show, the pointed leaf primordium *L* is rather easily distinguished from the blunt cluster primordium *C*. Barnard<sup>11</sup> states that the new organic apex of the bud arises from the apical tissue subtended by a leaf; he interprets this as a sympodial growth, so that the cluster primordium would be terminal. The sympodial origin of the cluster is given general support in the textbooks on viticulture. The rather equal division of the growing point occurring in some buds, the absence of a subtending leaf or stipular scale, and the alternate arrangement of leaves support this idea. A close examination of the growing points of our material revealed, however, that the division of the growing apex to form the cluster primordium in most buds is not equal, which suggests that the cluster may be a lateral rather than a terminal initiation. Further data will be required to support this view.

Goebel<sup>12</sup> states that he does not believe that tendrils are "formed as evident continuations of the internode below them and then only gradually pushed to the side by the stronger growth of the uppermost axillary shoot." Having usually found the tendril primordia situated distinctly laterally on the growing axis, he states: "They either from the first have the leaf-opposed position of the mature condition or . . . proceed from the apex of the axis itself through its unequal division." If the tendrils are not terminal and if, as Goebel points out, they are phyletically derived from inflorescences, it is not unreasonable to accept the possibility of the lateral initiation of cluster primordia, especially since there are many gradations between true cluster and true tendril, a fact that supports their homology. To us this conception appears more tenable than to accept the cluster as terminal and consider it a sympodial growth.

Field studies show that the tendrils occur in a leaf-opposed position the same as the clusters. They are never found below the clusters on the shoot. A tendril primordium (*T*) is shown in figure 9, plate 2, and figure 16, plate 3.

The divisions of the primordial cluster are first indicated by the appearance of bracts subtending the cluster branches (*B* in figs. 4 and 5, plate 1; and in fig. 11, plate 2). The first bract usually arises from the side of the cluster primordium farthest from the growing point. The first bracts were discernible a week or ten days after cluster initiation. By the middle of July, when the increase in size of the cluster primordia slows down, the lateral surface of the primordial clusters is crowded with branches, each subtended by a bract (fig. 9, plate 2, and fig. 18, plate 3). Although growing less rapidly as the season proceeds, the

cluster primordia divide again and again to give rise to secondary and tertiary cluster branches. When the buds open in spring, they are still in primordial form. The apical part of many clusters is still an undivided mass of meristematic tissue (figs. 16, 17, and 18, in plate 3). As shown in the apical part, *U* of figure 20, plate 4, differentiation continues just before and for a short time after the buds open. After the leafing out, however, it is soon superseded by the very rapid initiations of the flower parts.

### THE DEVELOPMENT OF FRUITFUL BUDS

The number of buds found to be fruitful for each position on the fifteen canes taken at each collection is shown in table 1. The columns, except the one at the left that indicates position, represent the fruitful buds

TABLE 1

NUMBER OF BUDS FOUND TO BE FRUITFUL OF THE FIFTEEN COLLECTED FROM VARIOUS POSITIONS ON THE CANES

Position of buds on cane	Date of collection								
	June 7, 1933	June 19, 1933	June 29, 1933	July 11, 1933	July 22, 1933	August 1, 1933	August 22, 1933	December 5, 1933	March 4, 1934
Basal	3	3	5	8	7	8	7	8	9
1	4	5	6	9	9	9	10	8	9
2	4	5	7	11	13	13	13	12	13
3	2	8	8	11	14	15	14	13	14
4	3	6	10	12	14	15	15	15	15
5	3	6	12	14	..	..	..	..	..
6	1	6	11	13	15	15	15	14	15
7	1	5	10	..	..	..	..	..	..
8	1	6	8	13	13	14	14	13	14
9	..	4	8	..	..	..	..	..	..
10	..	4	6	10	12	15	13	15	14
11	..	2	4	..	..	..	..	..	..
12	..	3	4	10	11	13	14	14	15
13	..	2	4	..	..	..	..	..	..
14	..	2	3	7	13	12	12	14	14
15	..	1	2	..	..	..	..	..	..
16	..	..	..	5	10	11	12	11	13
17	..	..	..	..	..	..	..	..	..
18	..	..	..	5	9	9	10	13	14
19	..	..	..	..	..	..	..	..	..
20	..	..	..	4	8	8	9	12	12

collected on the date shown at the top of the column. The number of differentiated buds increases rather rapidly as the season proceeds, up to about August 1. After this date there is a slow but continual increase in the fruitfulness of the buds above bud 12.

The buds on the basal end of a cane differentiate first. As the season advances, however, the maximum differentiation is soon shifted to the

region between the 4th and 12th buds of the cane, where it remains. The figures further indicate that the basal and first buds are the least fruitful of the buds on the part of the canes studied. The fruitfulness of the buds increased up to the 4th bud; from the 4th to the 12th buds it was about the same; from the 12th bud upwards it decreased. This observation closely agrees with crop records at Davis, which indicate that the total weight of crop, weight of cluster, and average crop per node increase from the basal up to the 6th bud. Between the 6th and 10th buds the figures remain about the same, whereas beyond the 10th bud they decline. The basal buds were 45 to 50 per cent fruitful, but the 6th to the 10th buds inclusive were 80 to 100 per cent fruitful. Keffer<sup>(10)</sup> reports

TABLE 2

## A KEY TO THE PHOTOMICROGRAPHS, SHOWING THE STAGE OF DEVELOPMENT OF THE BUDS AT DIFFERENT POSITIONS ON THE CANES

Date of collection	Basal node	1st node	4th node	6th node	10th node	14th node	20th node
June 7, 1933 . . . . .	..	..	..	1*	..	..	..
June 19, 1933 . . . . .	..	..	..	3	2	..	..
June 29, 1933 . . . . .	..	..	..	5	4	..	..
July 11, 1933 . . . . .	6	7	8	9	10	11	12
July 22, 1933 . . . . .	..	..	..	13	..	..	..
August 22, 1933 . . . . .	..	..	..	14	..	..	15
March 4, 1934 . . . . .	..	16	..	17	..	..	18

\* These numbers refer to the figure numbers of buds appearing in plates 1 to 3 inclusive.

similar results. He states: "The first buds formed in the spring are less well developed than the following buds; and . . . toward the end of the season buds on the distal end of the cane are not so well developed as those formed earlier in the season."

Table 1 shows the course of differentiation of the buds at a given node on the canes throughout the season. The lower buds on the cane were the first to show cluster initiation. In the buds farther up, other conditions being favorable, cluster initiation more or less paralleled the development of the shoot; that is, when the shoot had attained a given state of development, the buds began to show cluster initiation. The buds on the lower part (bud 4) of the cane reached maximum development before those in the midportion (bud 12) of the cane, the reason being the difference in the time of their formation.

The photomicrographs show the difference in size of primordial clusters at the different positions on the canes and at the different dates of collection. In order to show representative development, the specimens for the photomicrographs were so chosen (table 2) that the differentiation of a bud at a given position could be followed through the season,

as well as the differences in size of the primordial clusters in the buds at different positions on the canes for a given date. For the first purpose the 6th bud was selected and followed from the earliest collection showing cluster initiation until the next spring, to show the stages of growth of the primordial cluster. For the latter purpose the canes that were collected on July 11 were chosen. Such an arrangement of the buds reveals that for the 6th bud, the increase in size of the primordial cluster is rather rapid until the middle of July, then slows down gradually; after August the increase is relatively slow (figs. 1, 3, and 5 in plate 1; 9 in plate 2; 13, 14, and 17 in plate 3). The buds farther up on the cane, which developed later, were also later in differentiation and followed the 6th bud in this respect at each date of collection until after August 1. The most rapid increase in differentiation in these buds also came somewhat later in the season than that of the 6th bud. The uppermost buds examined were the latest in development in all respects. Not until the end of the season did their development begin to equal that of the 6th bud. In the upper buds of the canes, however, the development never did attain equality. A comparison of the 20th bud on the August 22, 1933 (fig. 15, plate 3) and March 4, 1934 (fig. 18, plate 3) collections will show that there was a marked increase in size during this period. The great increase in size, however, occurred prior to the October 6 collection. There was no perceptible change between the December 5 and March 4 collections.

Since the differentiation of buds on a cane starts from the base and proceeds upwards, a difference in the size of the primordial clusters in the same direction would be expected. An examination of the buds collected on July 11, 1933 (fig. 6, plate 1, and figs. 7-12, plate 2), shows that, although the first three buds are earlier in time of differentiation than the 4th to 10th buds, their primordial clusters are smaller. The 4th to 8th buds have the largest primordial clusters. Beyond the 8th bud the size of the primordial clusters decreased gradually, until in the 20th bud only the beginning of differentiation was visible. The trend of development in the buds of the August 22, 1933, collection was similar to that described above. The cluster primordia in the basal buds were smaller than those of the first and second buds. Their size increased gradually up to the 4th bud, became about constant from the 4th to the 10th bud, and above the 10th bud decreased again. By the time the buds were ready to open in spring these differences diminished. The differences that persisted, however, though small, were in the same direction as in the younger buds (figs. 16, 17, and 18, plate 3). The findings of Colby and Tucker<sup>10</sup> with Concord closely agree with these figures.

## SEQUENCE AND RATE OF DEVELOPMENT OF THE INFLORESCENCE

Our observations on the sequence of the development of the floral parts of *Vitis* agree with those of Sartorius,<sup>(1)</sup> Baranov,<sup>(2)</sup> and others—namely, that it is regular. The calyx, corolla, stamens, and pistil are differentiated in the order named. Each flower primordium pushes out from the axis, to which it is attached, as an undifferentiated, rather roundish mass of meristematic tissue (*Uf* in fig. 20, plate 4). The calyx (*S* in figs. 20 and 21, plate 4) first appears as a protuberance on either side of this meristematic surface in the longitudinal sections. The initiation of the corolla (*P* in fig. 21, plate 4) primordium follows the calyx in similar manner. As the calyx grows, the lobes bend inward, come in contact with each other, and give the impression of a coalescence. Snyder,<sup>(3)</sup> having observed a similar condition in *labrusca*, reports the case as an actual coalescence, while Sartorius<sup>(1)</sup> states that the end cells of the sepals are simply held together with a sticky substance in *Vitis vinifera*. Barnard and Thomas<sup>(4)</sup> could not find this condition in Sultanina. As the corolla lobes grow upward they separate the sepals (figs. 22 and 23, plate 4). During their upward growth the petals bend inward and come in contact with each other to form the so-called calyptra (*P* in fig. 22, plate 4).

According to Snyder<sup>(3)</sup> extensive cell division occurs in the parts of the petals that touch, and a considerable mass of what he terms "callus" is formed at their tips. This was not the case in our material. Usually the epidermis of petals has an irregular outline, and the cell walls bulge out. When the petals come together, the projecting cells of one intermesh into those of the other, and thus interlock the petals. This agrees with the findings of Sartorius.<sup>(1)</sup> The red-staining cuticle layer clearly shows the line of meshing; and the fact that the petals separate from each other at the base along this line when the calyptra is shed indicates the lack of actual union of the cells. Snyder's mass of "callus" cells is, in fact, a portion of the posterior lobe, which in sectioning has been left in the same plane with the two lateral lobes. The same red-staining substance aids in identifying the cells as belonging to the epidermal tissue. In this case the petal tissue is cut tangentially.

Before the calyptra is completely formed, the primordia of the stamens are discernible as definite lobes (*St* in figs. 21 and 22, plate 4).

The primordia of the carpels appear soon after the meshing of the petals (*Cp* in fig. 23, plate 4). They arise from the meristematic apex in a manner similar to the other parts. At this stage of development the stamens show no evidence of differentiation into anthers and filaments.

Figure 24, plate 4, shows the further development of the carpels; the stamens show the differentiation into anthers (*An*) and filaments (*F*). The primary sporogenous tissue is discernible in the anthers.

The further increase in size of carpels is associated with the development of the ovules. The ovules, their structures, and the sequence of their development were described in some detail by Berlese<sup>10</sup> as early as 1892. His descriptions have been confirmed and expanded by Sartorius,<sup>11</sup> Baranov,<sup>12</sup> and others. The sequence and rate of development of the structures of the ovule are described in greater detail by the latter workers. We have attempted to correlate the development of the individual flower and its parts with cluster size and time (table 3). The

TABLE 3  
LENGTH OF CLUSTER AND DEVELOPMENT OF FLOWER PARTS ON THE  
DATES ON WHICH THE INFLORESCENCES  
WERE COLLECTED

Length of cluster, inches	Date* of collection	Figure numbers (plates 4 and 5) showing average development for each group
1 1/2 - 2	March 18.....	19, 20
2 1/2	April 1.....	19, 21
2 1/2 - 3	April 8.....	22
3 - 4	April 13.....	23
4 - 5	April 21.....	24, 25
7 - 10	April 25.....	26, 27
	May 1.....	28, 29, 30

\* Vine development was almost three weeks earlier than usual during this period of development in the 1934 season.

rate of development of both the flowers and the cluster is influenced, however, by climatic and seasonal conditions.

Although the number of carpels in *Vitis* is two, it is not unusual to find three. Two ovules arise in each carpel. The ovule primordium (*Nu* in fig. 24, plate 4) first appears as a protuberance. It continues to increase in size until it completely occupies the ovarian cavity (fig. 25, plate 5). The placenta is axile. A ring of tissue, arising near the tip of the nucellar tissue, forms the inner integument (*I* in fig. 26, plate 5), which in turn is followed by the formation of a second ring of tissue outside the first, which develops into the outer integument (*O* in fig. 27, plate 5). Approximately a week after the outer integument is initiated, the megasporocyte has passed through the second meiotic division to form a tetrad. Since *Vitis* species have anatropous ovules, these structures must move through a considerable arc (figs. 26-29, plate 5). This growth begins soon after the inner integumentary ring becomes dis-

cernible (figs. 26 and 27, plate 5). At this stage the macrospore mother cell can be seen. The bending of the funiculus continues until the ovule tip is directed downward toward the placenta (fig. 29, plate 5). The inner integument has now grown to enclose the nucellus entirely, leaving only the micropylar opening at the lower end. In the Sultanina the development of the inner integument is abnormal, so that its tubular tip is distorted and presses against the ovary wall toward the funiculus (fig. 30, plate 5). Pearson,<sup>10</sup> who observed a similar condition, states that the outer integument is abnormally short. By the time the stage of development shown in figure 30 is reached, the egg cell is ready for fertilization.

During these stages of megasporangial development the primary sporogenous tissue in the anthers divides to form microspore mother cells. About the time the first integumentary ring appears in the ovule, the microspore mother cells enter the prophase of the first meiotic division. At the stage of development shown in figures 28 and 29 (plate 5) each member of a tetrad rounds off and separates to form a microspore. By the time the ovule has reached the stage of development shown in figure 30, the microspores have become mature.

#### SUMMARY

A histological study of the Sultanina was undertaken in order to determine the time of differentiation of the fruit buds and the course of development of the primordia.

Cluster primordia begin to be initiated during the first week of June. They appear as blunt, rather broad outgrowths of the growing point of the bud. The leaf primordium, on the contrary, appears as a pointed outgrowth from the growing point and is readily distinguished from the cluster primordium.

The most productive part of the canes is the portion between the 4th and 12th buds. The basal and distal buds on a cane are the least productive. The primordial clusters in the basal and apical buds of the canes do not become so large as those in the buds in the middle of the canes.

The differentiated cluster primordia increase rapidly in size during the early season and then slow down. There is no perceptible increase during the dormant period.

The formation of a bract is the first indication of the division of the primordial cluster. Bract formation is discernible a week or 10 days after cluster initiation. Lateral cluster branches arise in the axils of these bracts. By the end of the season the lateral surface of the primordial cluster is a mass of bracts and branch primordia.

Tendril primordia form later in the season than primordia of the clusters.

The development of the flower is regular. The parts follow each other in rapid succession in their development. Six to seven weeks after leafing out, the development of parts is complete.

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## **EXPLANATION OF PLATES**

## PLATE 1

Photomicrographs of longitudinal sections through the buds from the collections of June 7, 19, 29, and July 11. ( $\times 26$ .) Compare figures 1, 3, and 5 with figure 9, plate 2, and with figures 13, 14, and 17 of plate 3, to note the influence of time on stage of development; and compare figure 6 with figures 7-12, plate 2, to note the influence of position of the bud on the stage of development of the cluster primordium.

Fig. 1.—Bud from the 6th node, June 7 collection. *C*, Early stage in the development of the cluster primordium. *L*, Leaf initial.

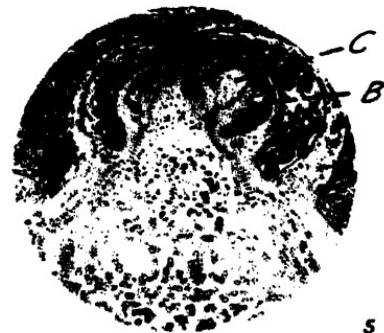
Fig. 2.—Bud from the 10th node, June 19 collection. *C*, Early stage in the development of the cluster primordium. *L*, Early stage of leaf development.

Fig. 3.—Bud from the 6th node, June 19 collection. *C*, Cluster primordium. *A*, Buds in the axils of the primordial leaves.

Fig. 4.—Bud from the 10th node, June 29 collection. *C<sub>1</sub>*, Early stage in the development of the upper (second) cluster primordium. *C*, Lower (first) cluster primordium. *B*, Initial stage of a bract on the primordial cluster. *A*, Buds in the axils of the primordial leaves.

Fig. 5.—Bud from the 6th node, June 29 collection. *C*, Cluster primordium. *B*, Initial stage of a bract on the primordial cluster.

Fig. 6.—Bud from the basal node, July 11 collection. *C*, Cluster primordium.



## PLATE 2

Photomicrographs of longitudinal sections through buds from different positions on the canes from the collection of July 11. ( $\times 26$ .) Compare figures 7-12 with figure 6, plate 1.

Fig. 7.—Bud from the 1st node. *C*, Cluster primordium. *L*, Early stage of leaf development. *A*, Buds in the axils of the primordial leaves.

Fig. 8.—Bud from the 4th node. *C*, Cluster primordium. *Cb*, Branches on the cluster primordium.

Fig. 9.—Bud from the 6th node. *C*, Cluster primordium. *Cb*, Branch on the cluster primordium. *T*, Early stage of development of a tendril. *B<sub>1</sub>*, Bract on the branch primordium. *B*, Bract subtending the branch primordium.

Fig. 10.—Bud from the 10th node. *C*, Cluster primordium. *B*, Bract subtending the branch primordium.

Fig. 11.—Bud from the 14th node. *C*, Cluster primordium. *B*, Initial stage of a bract on the primordial cluster.

Fig. 12.—Bud from the 20th node. *C*, Cluster primordium. *L*, Early stage of leaf development.



7



8



9



10



11



12

### PLATE 3

Photomicrographs of longitudinal sections through buds from the collections of July 22, August 22, and March 4. ( $\times 26.$ )

Fig. 13.—Bud from the 6th node, July 22 collection. *C<sub>1</sub>*, Upper cluster primordium. *C*, Lower cluster primordium. *Cb*, Branch on the cluster primordium. *B*, Bract subtending the branch primordium.

Fig. 14.—Bud from the 6th node, August 22 collection. *C*, Cluster primordium.

Fig. 15.—Bud from the 20th node, August 22 collection. *C*, Cluster primordium. *B*, Bract subtending the branch primordium.

Fig. 16.—Bud from the 1st node, March 4 collection. *C*, Cluster primordium. *T*, Early stage of development of a tendril.

Fig. 17.—Bud from the 6th node, March 4 collection. *C*, Cluster primordium. *Cb*, Branch on the cluster primordium. *B*, Bract subtending a branch primordium.

Fig. 18.—Bud from the 20th node, March 4 collection. *C*, Cluster primordium. *Cb*, Branch on the cluster primordium. *B<sub>1</sub>*, Bract on the branch primordium. *B*, Bract subtending a branch primordium.



#### PLATE 4

Photomicrographs of longitudinal sections through cluster branches, showing the initiation and development of the flower parts.

Fig. 19.—Cluster branch about two weeks after leafing out. ( $\times 26$ .) *K*, The coming together of the petals to form the calyptra. *B*, Bract subtending an individual flower. *R*, The coming together of the sepals, which occurs early in the development of the flower parts.

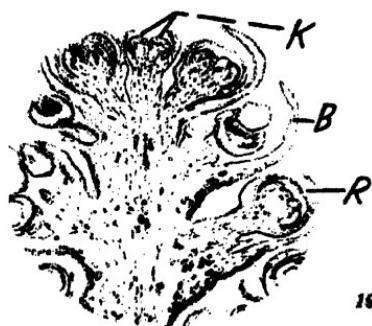
Fig. 20.—Cluster branch about one week after leafing out. ( $\times 100$ .) *U*, Undifferentiated mass of meristematic tissue from which several more flowers may arise. *Uf*, Undifferentiated mass of meristematic tissue from which the flower parts will arise. *S*, Beginning of calyx differentiation.

Fig. 21.—A flower about two weeks after leafing out. ( $\times 100$ .) *S*, The lower line shows an early stage of calyx development, while the upper line points to a later stage of development of the calyx. *P*, Early stage of corolla development. *St*, Initiation of stamen differentiation.

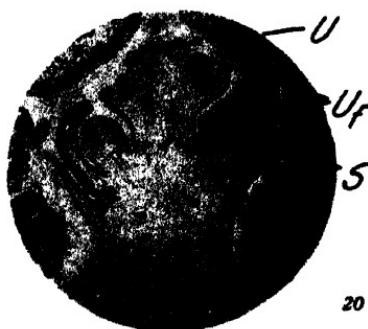
Fig. 22.—A flower about three weeks after leafing out. ( $\times 100$ .) *S*, Calyx; the sepals still appear coalesced. *P*, Corolla; the petals are coming together above to form the calyptra. *St*, Initiation of stamen development.

Fig. 23.—A flower three to four weeks after leafing out. ( $\times 100$ .) *S*, Calyx. *P*, Corolla. *St*, Stamen. *Cp*, Initiation of carpel development.

Fig. 24.—A flower about four weeks after leafing out. ( $\times 100$ .) *P*, Calyx. *S*, Corolla. *An*, Anther. *Cp*, Carpel. *F*, Filament. *Nu*, Nucellus.



19



20



21



22



23



## PLATE 5

Photomicrographs of longitudinal sections through flowers, showing the development of the parts. ( $\times 100$ .)

Fig. 25.—A flower four to five weeks after leafing out. *Ov*, Ovary. *Nu*, Nucellus. *F*, Filament.

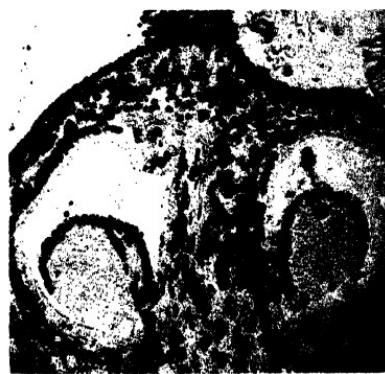
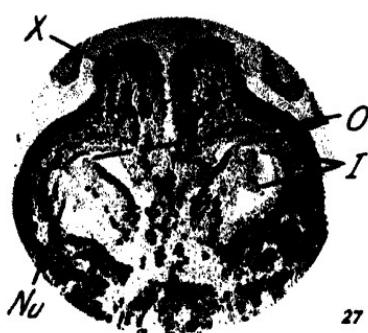
Fig. 26.—A flower several days later than that shown in figure 25. *Nu*, Nucellus. *I*, Initiation of the inner integument.

Fig. 27.—A flower about a week later than of figure 25. *X*, Early stage in the development of the style. *O*, Outer integument. *I*, Inner integument. *Nu*, Nucellus. (The bending of the funiculus is first discernible at this stage.)

Fig. 28.—A flower several days later than that of figure 27. The ovule is pointing downward, and its parts have advanced in development. *X*, Style. *O*, Outer integument. *Nu*, Nucellus. *I*, Inner integument.

Fig. 29.—A flower five to six weeks after leafing out. The flower parts are approaching maturity.

Fig. 30.—The mature megasporangium. The inner integuments elongate abnormally, so that the micropyle may be contorted. *M*, Micropyle. *O*, Outer integument. *I*, Inner integument.





# **MORPHOLOGY OF THE FLOWER AND FRUIT OF THE LOQUAT**

**ROBERT M. SMOCK**



# MORPHOLOGY OF THE FLOWER AND FRUIT OF THE LOQUAT<sup>1,2</sup>

ROBERT M. SMOCK<sup>3</sup>

## INTRODUCTION

THE LOQUAT (*Eriobotrya japonica*), indigenous to China, is grown more or less extensively in California, Florida, and the Gulf States. *Eriobotrya japonica* is in the family Rosaceae, subfamily Pomoideae. The Greek translation of *Eriobotrya*—"woolly inflorescence"—well depicts the extremely hairy condition of buds, flowers, fruits, and leaves. In China the loquat is called "rush orange."

Bailey<sup>"4</sup> describes the tree as small and evergreen with leaves "elliptical to oblong-ovate, nearly sessile, and remotely toothed." The small, white flowers are borne in woolly panicles 4 to 7 inches long (fig. 1). Development of the panicle is acropetal. The flower panicles are terminal on the current season's growth. Growth extension occurs from terminal leaf buds on nonfruiting branches and from the distal lateral leaf bud on fruiting branches. The flowers are pentamerous, and each of the five carpels contains two ovules; ordinarily only one to eight seeds develop. Seedless varieties, though sometimes reported, are of no commercial importance. Condit<sup>(5)</sup> has described climatic adaptations, culture, and varieties of the loquat.

This paper presents the results of a study of the morphology of the loquat flower and fruit.

## MATERIALS AND METHODS

Loquat buds, flowers, and fruits were collected at weekly intervals during the 1934-35 season from a tree of the Advance variety on the University Farm at Davis, California.

The extremely hairy condition of the buds made paraffin sectioning difficult. Celloidin, double infiltration as described by De Zeeuw,<sup>(6)</sup> butyl alcohol, and glycerine-butyl alcohol were therefore employed as softening agents in attempts to avoid tearing of sections. Although fairly satisfactory results were obtained with celloidin-imbedded material, the

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<sup>2</sup> The author wishes to express his appreciation for the help and criticism of Dr. Warren P. Tufts, who suggested the problem.

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<sup>4</sup> Superscript numbers in parentheses refer to "Literature Cited" at the end of this paper.

method adopted was to kill bud samples in formalin-acetic-alcohol fixative and then expose them for several hours to 5 per cent hydrofluoric acid in 70 per cent alcohol.

Flowers and fruits were scraped practically free of hairs with a scalpel before being exposed to the killing agent. Soaking of the paraffin blocks



Fig. 1.—Panicle of loquat flowers at anthesis.

in water in an oven at 30° centigrade for several weeks facilitated sectioning.

Medium Flemming's killing agent and Navashin's modification of Karpechenko's were employed for flowers used in macrosporogenesis studies. The former proved more satisfactory. Delafield's haematoxylin stain was used for most of the material; gentian violet or Haidenhain's

haematoxylin for more delicate work. Although sections were usually cut 14 microns thick, flower sections used in macrosporogenesis studies were 8 microns in thickness.

#### FLOWER-BUD DEVELOPMENT

The period of flower-bud differentiation and of flowering is extended. Buds showing early stages of flower-bud development were collected as late as November 6. Buds were not collected early enough in 1934 to indicate at what time of year the first signs of differentiation began.

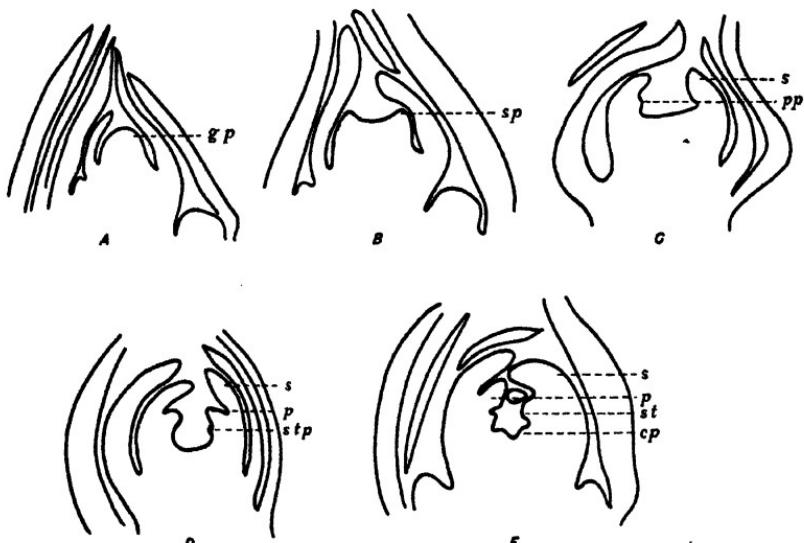


Fig. 2.—Sections showing development of typical loquat flower bud. The parts are: *gp*, growing point; *s*, sepal; *p*, petal; *st*, stamen; *sp*, sepal primordium; *pp*, petal primordium; *stp*, stamen primordium; *cp*, carpel primordium.

Buds that possessed flower primordia were tagged on October 17; these were in full bloom by December 28. The period of full bloom for the whole tree was from October 17 to February 1.

The first discernible sign of flower-bud differentiation is a distinct, blunt protuberance. As a result of a rapid multiplication of the cells at the outer edge of this protuberance, a slightly elevated ridge appears (fig. 2, *A*). Very soon cell division at five points around the circumference of this ridge results in the formation of the sepal primordia (fig. 2, *B*). The torus, meanwhile, has been undergoing rapid development, especially basal to the calyx. As a result of this toral growth the rapidly forming sepals are elevated. They develop quickly, and their apices grow towards each other so as to enclose the distal portions of the carpels. The

sepals as well as all of the exposed toral surfaces are densely covered with long epidermal hairs.

Very soon after the sepal primordia develop, those of the petals appear, arising in a whorl alternating with the sepals (fig. 2, C). Growth of the petals and sepals continues, and soon they cover over in "hood" fashion the cup-shaped torus (figs. 2, D and 2, E). Each petal is constricted at the point of divergence from the torus.

Shortly after the petal primordia appear, those of the stamens develop in three cycles around the torus directly inside the petals. The outer cycle of stamens develops first; then the middle, and finally the inner cycle is initiated. The innermost and middle cycles of stamen primordia number five each, while those of the outer consist of ten, there usually being twenty stamens in all. The stamen cycles are too close together to be easily differentiated from one another by the naked eye.

The five carpel primordia are formed soon after the inner cycle of stamen primordia is developed. They arise from the cup-shaped receptacle just basal to the inner cycle of stamens and some distance from the center of the torus (that is, at the apex of the pedicel). Infolding of the carpel edges takes place very early in carpel development. Carpel growth proceeds rapidly, and soon adnation of the lower portions of the carpels is so complete that one cannot discern the infolded edges, which are clearly distinguished only in the upper half of the flower. The styles are completely free. The ovules arise from the infolded carpel edges which form the placentae. The exposed surfaces of the carpels develop a very dense pubescence (plate 1).

During the development of the carpels the torus continues to grow, and thus elevates the stamens, petals, and sepals. The tip of the axis is below the carpels, and the placentae are carpel tissue only. Since the center of the torus does not undergo any growth, there is no central column of axis tissue extending up through the set of five carpels.

As the distal portions of the ovaries remain exposed and are not covered with receptacle tissue, the loquat flower is only partially epigynous. A photomicrograph of the developing floral parts is shown in plate 2.

#### OVULE DEVELOPMENT AND MACROSPOROGENESIS

Early in carpel development there occurs, slightly below the middle of each carpel, a growth of the placenta which develops into the ovule. Soon a second protuberance is seen—directly basal to the first—which becomes the obturator. Although the obturators are relatively large, their later growth does not keep pace with that of the ovules; at the time of full bloom the obturator is scarcely discernible to the naked eye.

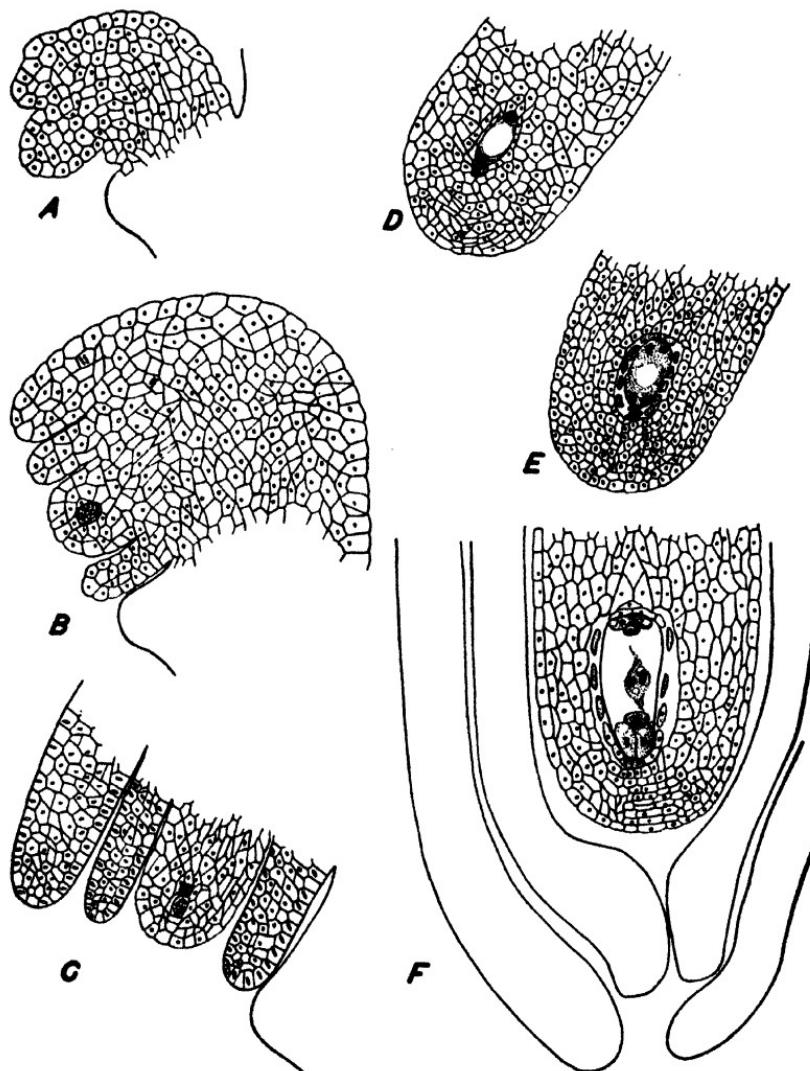


Fig. 3.—Macrosporogenesis in the loquat. *A*, Development of integuments; *B*, macrospore mother cell just before reduction division; *C*, formation of linear tetrad of macrospores; *D*, two-celled gametophyte and degenerating macrospores; *E*, four-celled gametophyte and degenerating nucellar cells, contiguous to embryo sac; *F*, mature macrogametophyte.

The two ovules in each carpel are usually side by side, one being slightly more elevated than the other. As growth of the young ovule continues, there is more cell division on one side than on the other, and soon the micropylar end points downward and outward toward the car-

pel wall, later becoming typically anatropous. At about the time when the ovule is at a right angle to the funiculus, first signs of the inner integument appear. From the epidermis of the ovule at a level just basal to a point where the macrospore mother cell will later develop arises the inner integument (fig. 3, A). Very soon thereafter the outer integument originates from the epidermal layer just basal to the point of origin of the inner integument. The outer integument grows somewhat faster than the inner; and, by the time the female gametophyte is fully developed, the integuments fully enclose the nucellus.

Soon after the appearance of the initials of the inner integument, the macrospore mother cell may be observed. Its origin was not noted although an archesporial cell was observed in a few instances. The macrospore mother cell usually lies two to four cells back from the micropylar end of the nucellus. Soon the macrospore mother cell undergoes heterotypic division. A chromosome plate in such a cell during heterotypic division is seen in figure 3, B. Thirty-two chromosomes were counted in this plate. To ascertain definitely the chromosome number, more such plates would be necessary. Moffett<sup>(6)</sup> has reported that root tips of the loquat had thirty-four chromosomes, and it is possible that two chromosomes were not visible in the plate counted. Loquat chromosomes are very small and are subject to clumping during fixation.

Heterotypic is followed immediately by homeotypic division, and a linear set of four macrospores is formed (fig. 3, C). The chalazal macrospore is functional, and the other three disintegrate (fig. 3, D). No irregularities could be seen in the formation of the eight-nucleate female gametophyte. During the development of the embryo sac there is a dissolution or breaking down of cells of the nucellus contiguous to the embryo sac. This dissolution process, which is concomitant with sac development, seems to allow for volume increase of the sac. A conspicuous feature of the nucellus when the mature female gametophyte is formed is a group of large, elongated cells at the chalazal end of the embryo sac (figs. 3, E and F).

Tetrads of microspores could be observed during the formation of the macrospore mother cell. When the linear tetrad of macrospores was formed, the microspores were still in the tetrad stage. During the formation of the two-celled macrogametophyte, pollen was observed in the anthers.

The curvature of the nucellus increases during the development of the macrogametophyte; and by the time the eight-nucleate state is attained, the ovule is completely anatropous. The micropylar end rests against the obturator.

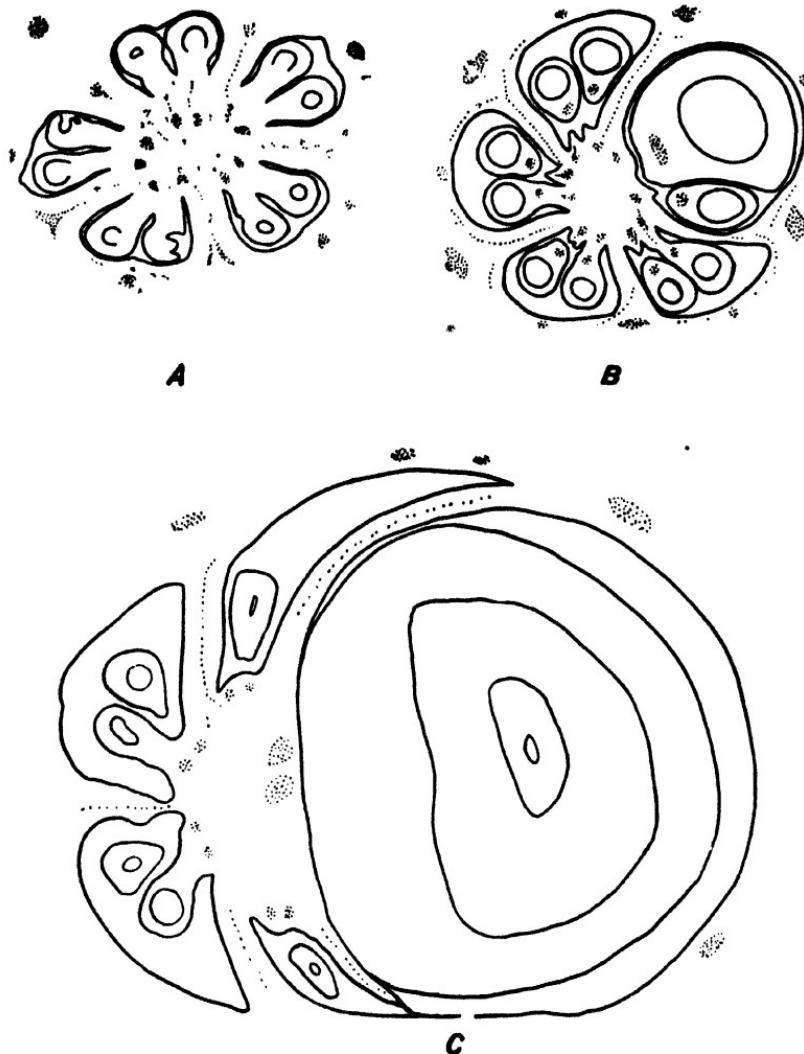


Fig. 4.—Development of seed in loquat and abortion of ovules. A, Ten potential seeds; B, initiation of abortion of nine ovules; C, seed and aborted ovules (note distortion of carpel walls due to growth by seed).

#### OVULE ABORTION

Apparently all ten ovules develop normal gametophytes. It was not discerned whether the eggs in all ten ovules are fertilized or not; however, in this variety only one or two ovules develop embryos. The cause of abortion of eight or nine ovules has not been determined. Climatic

influence on the nutritional status of the ovules may have some importance, however, since in the more southern portions of the state four or five seeds often develop in the variety Advance. In the ovules which failed to develop embryos the gametophytes remained apparently normal for several days and then degenerated. Two functional seeds seldom develop in the same carpel; but, where they do, one soon supersedes the other, and only one seed develops in the fruit. The functional seed or seeds develop very rapidly and soon distend the confines of their carpel so that the other carpels are distorted and compressed into a very small area. Degrees of abortion are illustrated in figure 4, A, B, and C.

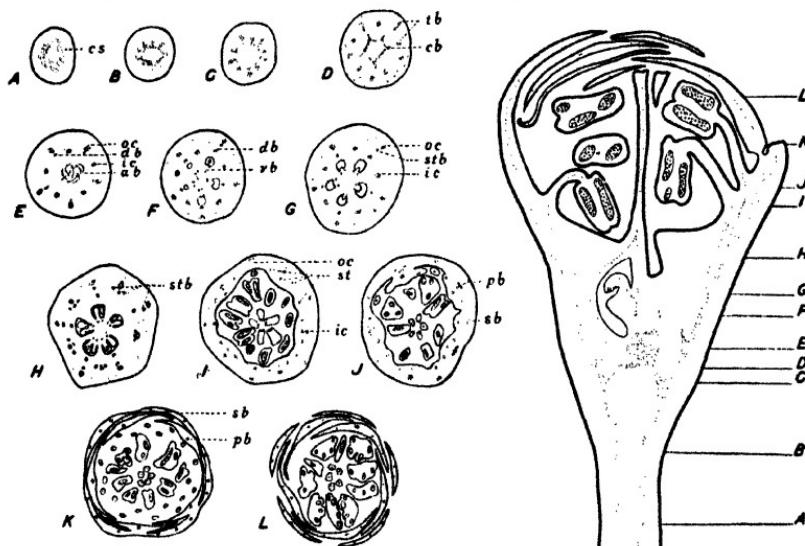


Fig. 5.—Vascular anatomy of loquat flower. Diagram on right shows levels at which depicted sections were cut. The parts are: *cs*, central stele; *cb*, central bundles; *tb*, toral bundles; *db*, dorsal bundles; *ab*, anastomosing bundles; *stb*, stamen bundles; *sb*, sepal bundles; *oc*, outer-cycle primary bundles; *ic*, inner-cycle primary bundles; *vb*, ventral bundles; *pb*, petal bundles.

### VASCULAR ANATOMY OF FLOWER AND FRUIT

The vascular anatomy of a typical terminal flower is described in this report. Terminal flowers on the panicle have longer pedicels, and the method of bundle divergences is easier to trace in detail than in the pedicels of lateral flowers.

At the base of the pedicel the vascular cylinder is complete and more or less pentagonal (fig. 5, *A*). As it is traced up through the pedicel, however, it is seen to divide into ten distinct bundles (fig. 5, *B*). The exact point where this division occurs varies with different flowers, but is

usually about midway up the pedicel. The ten bundles diverge outward and are soon more or less equidistant, and the smaller bundles remaining between them diverge toward the median axis of the pedicel (fig. 5, C). These smaller bundles in the median portion form a star-shaped column just distal to the apex of the pedicel (fig. 5, D). The ten major bundles at this point form two cycles of five each, every alternate bundle being in the same cycle. At the apex of the pedicel they diverge into the toral region of the flower and follow the region between the pith and cortex.

Just basal to the carpels, the small bundles in the inner cylinder or median portion undergo extensive anastomosis and invade the central pith area to a considerable extent (fig. 5, E). This anastomosis continues to a level about even with the base of the carpels. Above this point the bundles become discrete. Two of these distinct small bundles extend up through each carpel and form the placental bundles of the flower. These ventral bundles furnish the vascular supply to the ovules and fuse before extending into the styles.

As has been stated, the ten primary or toral bundles follow the line of demarcation between the pith and the cortex. The bundles of the outer cycle are opposite the dorsal sutures of the carpels, and those of the inner cycle alternate with the carpels. At a level just below the bases of the carpels, five small bundles diverge from the outer cycle of toral bundles (fig. 5, E). All five do not branch from the primary bundles at exactly the same level. These five bundles (the dorsals) diverge toward the center of the flower, closely follow the dorsal sutures of the carpels, and then pass through the distal portions of the carpel walls and up into the styles (fig. 5, F). Thus there are fifteen main vascular bundles in the carpillary system—ten ventrals and five dorsals.

From each of the five primary bundles of the outer cycle arises one branch from the inner or pith side at a point on a level with the bases of the ovules (fig. 5, G). Although the exact point of origin of these branches varies, none branches at a point higher than at a level even with the median portion of the ovules. These five branches continue upward through the torus and terminate, one in each stamen of the inner cycle. At a point somewhat distal to the point of origin of the bundles that terminate in the inner stamen cycle, one bundle branches off from each of the inner cycle of primary toral bundles (fig. 5, H). These branches extend upward and terminate in the middle cycle of stamens. Slightly higher than the point of origin of the last-mentioned bundles, two more branches are given off from each of the inner cycle of primary toral bundles. These branches constitute the vascular supply of the outer cycle of stamens (fig. 5, H).

The petals derive their vascular supply from the inner cycle of primary bundles. After divergences for both the middle and outer cycle of stamens have been given off, each bundle of the inner cycle continues up through the cup-shaped torus (above the carpel level) and breaks into three separate bundles. The innermost of these three diverge into the petals, and the outer two extend into the sepals, as will be described later (fig. 5, K). The bundle extending into the petals forms the midrib and gives off branches to form a vascular network.

The sepals obtain their vascular supply from both the inner and the outer cycle of toral bundles. The median bundle or midrib of each sepal is the termination of one of the outer toral primary bundles. The small lateral bundles in the sepals are divergences from the inner primary bundles mentioned above. Following the divergences into the petals by one of the three bundles described in the foregoing paragraph, the second extends into a sepal to the right, and the third into the sepal to the left of the adjacent petal (fig. 5, K).

The cortex of the flower and fruit gains its vascular supply from branches from the ten primary toral bundles. These branches divide comparatively little until they reach the subepidermal region, where they branch and anastomose profusely, forming a network of fine bundles.

The vascular anatomy of the loquat resembles that of the apple as described by Kraus and Ralston<sup>10</sup> but differs in several respects. The anastomosis occurring in the median portion of the stele of the pedicel does not extend through so long a distance in the loquat as in the apple. Divergences for the stamen bundles occur at considerably lower levels in the loquat than in the apple.

#### DEVELOPMENT OF THE FRUIT

After fertilization, the loquat fruit develops very rapidly. The first indication of fruit enlargement is a thickening of the toral rim immediately above the carpel level. The whole toral region undergoes cell division and enlargement more or less uniformly throughout. The sepals grow toward the center and cover over in "hood" fashion the distal portions of the carpels. The sepal bases thicken and persist as permanent structures, whereas the petals, stamens, and styles dry up. The hood or cap enclosing the distal portions of the carpels may be excised from immature fruits, and the five carpels be exposed to view (plate 4, A). Remnants of the petals, stamens, and styles may be seen when this toral rim is cut off.

The toral rim of a number of fruits was removed on April 11, 1935, while the fruits were still on the tree. When the cut was made above the

carpels, the torus was almost completely regenerated, and the carpels were again hooded over; but whenever the cut pierced the carpel proper and cut a portion of the ovule, the fruits either died or ripened prematurely without attaining their full size.

The functional ovule develops into a fertile seed occupying the whole central region of the fruit; and the confines of the individual carpel walls which surrounded nonfunctional ovules are wholly disrupted (plate 3, *A* and *B*). The distal wall of the carpel in which the functional seed is found undergoes considerably more growth than do those of the other carpels. The result is an elevation of the distal wall of the carpel containing the functional ovule over those of the others.

By the time the fruit is mature, the carpel walls are more or less parchmentlike. The seed is extremely hard, the integuments and cotyledons being very resilient.

The skin of the mature fruit is comparatively tough, being more leathery than that of either the pear or the apple.

As has been indicated in the foregoing discussion, the edible portion of the loquat is entirely toral in nature, consisting of the pith and cortical areas. Development of the edible portion consists of a rather uniform growth of receptacle tissue throughout the fruit (plate 4, *C*). The toral cells of the mature fruit are large, thin-walled, and very juicy.

#### EFFECT OF LOW TEMPERATURES ON DEVELOPING LOQUAT FRUITS

Since the flowering period at Davis in the 1934-35 season was from the middle of October to February, the flowers and young fruits were subjected to comparatively low temperatures. The mean temperature of December was 48.3° F., and that of January, 45.8°; but the flowers not only were uninjured but were fertilized and developed into fruits during this period. Late in January the temperature on one night dropped to 29°. This resulted in an injury to many young fruits, thinning numerous panicles to one fruit. In nearly all cases, the terminal fruits persisted.

Many fruits injured by this cold period did not drop from the tree. In them the toral region became loosened from the carpels and could easily be "shelled" off. When the ovules of these injured fruits were not affected, these loosened areas tightened within a few weeks, and the fruits grew to normal size. The fruits that had injured ovules and yet did not drop never recovered from the injury to the torus; they grew very little in size thereafter and ripened prematurely.

## SUMMARY

Floral development of the loquat (*Eriobotrya japonica*) is acropetal. The development of the floral organs is here described in detail. The loquat flower is semiepigynous and possesses ten ovules.

In the development of the macrogametophyte the macrosore mother cell undergoes heterotypic and then homeotypic division, resulting in the formation of a tetrad of macrosores. The chalazal macrosore only is functional, the remaining three disintegrating. As a result of three successive nuclear divisions, an eight-nucleate female gametophyte is formed.

Apparently normal macrogametophytes are formed in all ten ovules, but in the variety Advance only one or occasionally two seeds develop in each fruit at Davis, California.

The vascular anatomy resembles that of the apple, but the region of anastomosis in the stele of the pedicel is less extended than in the apple. Also, the point of divergence of the stamen bundles occurs at a considerably lower level in the loquat than in the apple.

The fruits have a hood or cap of toral tissue enclosing the distal portion of the ovary. This toral cap may be excised, and the distal portions of the carpels exposed to view.

The functional seed or seeds are fertile and occupy the whole central region of the fruit. Their development results in a marked distortion of the carpel walls and a complete dislocation of the nonfunctional ovules and the carpels in which they were located.

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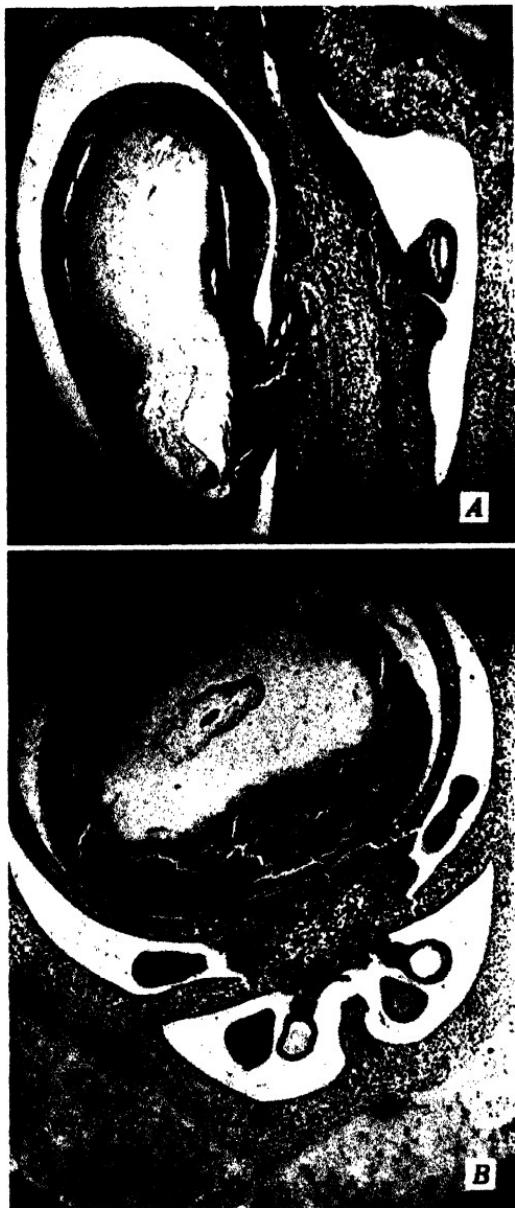




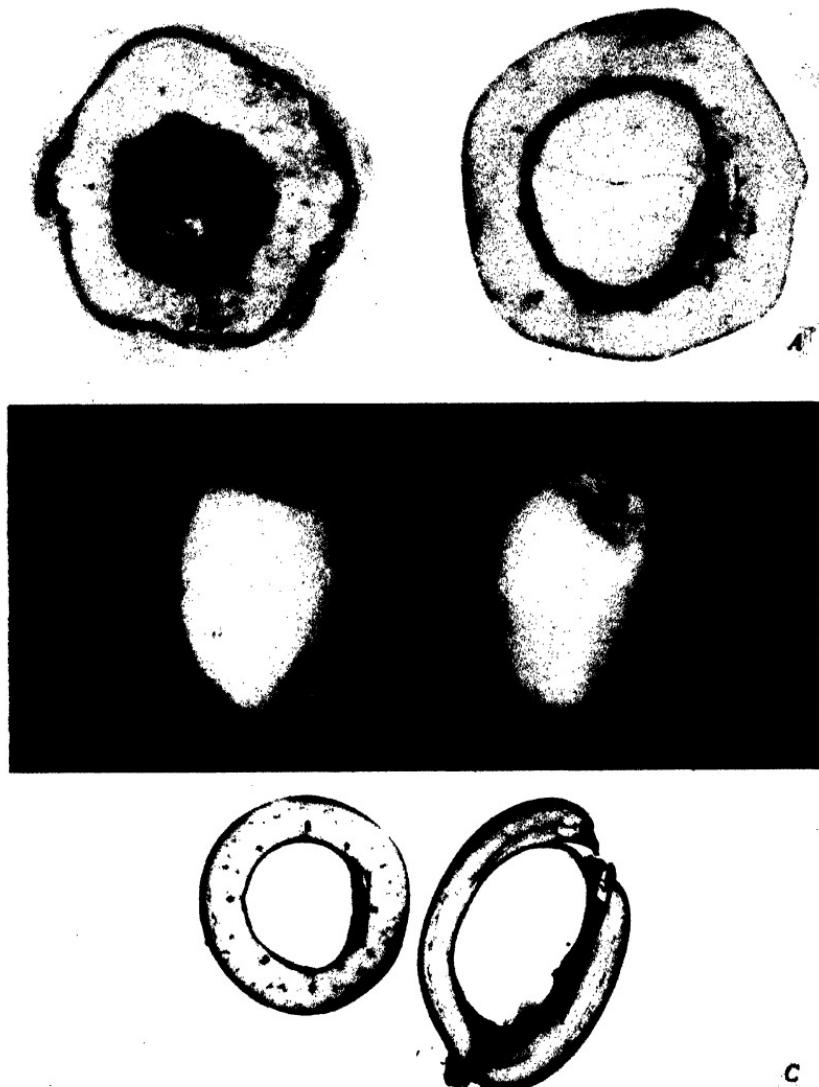
Photomicrograph of very young loquat flower. Note extreme hairiness of bracts  
and of the young flower.



A, Photomicrograph of developing floral parts of the loquat.  
B, Photomicrograph of two-nucleate female gametophyte.



A. Photomicrograph of longitudinal section of young fruit, showing developing seed (at left) and aborting ovules (at right). B. Cross section of young fruit showing developing seed and aborting ovules.



A. In the fruit on the left, the toral rim has been cut to expose the carpels. The fruit on the right has been cut to show the seed and the distorted carpels. B. Mature loquat fruits of the Advance variety. These fruits are golden yellow. C. Cross and longitudinal cleared sections of loquat fruits. The seed has been removed in each case. The conspicuous and edible portion of the fruit is entirely toral in nature. The primary toral bundles in the cross section indicate the line of demarcation between pith and cortex. The compressed carpel walls may be seen in both sections.





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